# STUDIES ON THE DISEASE COMPLEX OF BALSAM CAUSED BY *MACROPHOMINA PHASEOLINA* AND *MELOIDOGYNE JAVANICA*

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BY

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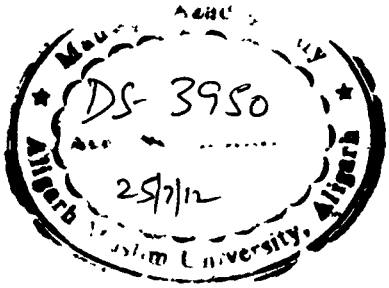
*Dedicated*

*To*

*My (Late) Grand Father*

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# *Certificate*

*This is to certify that the work presented in this dissertation entitled "**Studies on the Disease Complex of Balsam Caused by Macrophomina phaseolina and Meloidogyne javanica**" is an original piece of work carried out by* **Miss. Shweta Sharma** *under my guidance and supervision and has not been submitted elsewhere for the award of any other degree and can be submitted in partial fulfilment of the requirements for the award of the degree of **Master of Philosophy in Botany** (**Plant Pathology**).*

(Tabreiz Ahmad Khan)
Supervisor

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Shweta

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# *Introduction*

# INTRODUCTION

Balsam (*Impatiens balsamina* L.) is native to Southern and South East Asia and found throughout the tropical and subtropical parts of India. It is an annual plant has red, pink, purple or white flower and reaches to a height of 1-3 feet. In India it is mostly grown as an ornamental plant. Moreover, different parts of the plant are used to treat the diseases of human being. In China, the aerial parts are used for the treatment of auricular rheumatism, bruises and beri - beri. In South Korea, the plant is used in treating tuberculosis, carbuncles and dysentery. The methanolic extract of the whole plant yields a naphthoquinone derivative, 2-methoxy-1, 4 naphthoquinone which exhibited strong antifungal activity against *Candida albicans*, *Aspergillus niger*, *Cyrtococcus neoformans* and *Epidermophyton floccosum*; the activity was comparable to that of nystatin. The extract also showed strong antibacterial activity against *Bacillus subtilis* as well as *Salmonella typhimurium*. The plant extract also inhibited the growth of Potato Virus Yon chillies. In Brunei, decoction of roots is given in irregular menstruation. The plant is reported to contain cyanophoric constituents antibacterial substances. In Japan, the juice obtained from the white petals is applied topically to treat several types of dermatitis including urticaria. An ethanolic extract (35%) of flowers shows significant anti-anaphylactic activity in mice.

Plant parasitic nematodes, among the soil biota, form a separate group constituting a significant component (about 12% of soil microflora and fauna) of the soil ecosystem. Plant parasitic nematodes are capable of producing recognizable disease symptoms on suitable susceptible hosts. Most of the diseases caused by nematodes are detrimental. Endoparasitic nematodes are more damaging and agriculturally important than other groups. Plant parasitic nematodes affect the production and economy of crop in diverse ways such as reduction in quality and quantity of crop, need of additional fertilizer and water, application of nematicides and impediment of production and trade by phytosanitary regulations(Weischer,1968). Root – knot nematodes are among the most economically destructive group of plant parasitic nematodes causing damage and yield losses on most of the cultivated plants (Sasser and Freckman, 1987).

There are numerous estimates of economic importance of nematodes in crop production on a world wide and individual country basis, but precise values cannot be

determined. It was because of nematodes "small size and hidden way of life" and lack of definite information on its occurrence and pathogenicity. There are some reports of crop losses in terms of money. A loss of 5 millon kroners was estimated due to cereal cyst nematode, *Heterodera avenae* in Denmark (Stapel, 1953). USDA estimated an annual crop loss of 372,335000 dollars to sixteen crops (Taylor, 1967). In other estimates, Hutchinson *et al.* (1961) and Cairns (1955) reported loss of $ 250 million and $ 500 million due to nematodes. Southey and Samuel (1954) reported a crop loss to the extent of 200,000 tonnes of potato annually by *Globodera rostochiensis* in England and Wales. The estimated annual loss due to nematodes in USA was of the order of $10, 38, 374,300 in 16field crops, $ 225,145,900 in fruits and nut crops, $ 266,289,100 in vegetable crops and $ 59,817,634 in ornamental crops (Feldmesser *et al.*, 1971). Mc Gregor (1978) from U.S.A. attributed a yield loss of $ 320 million due to *Heterodera zeae.* Yield losses of 20-59% have been reported to cowpea (Ogunfowora, 1987). Sasser and Freckman (1987) have indicated annual crop losses due to plant parasitic nematodes on worldwide basis to the tune of $ 100 billion. In a worldwide survey conducted by Sasser (1989), the most important genera of plant parasitic nematodes revealed were *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus, Globodera, Tylenchulus, Xiphinema, Radopholus, Rotylenchulus* and *Helicotylenchus.* This order of importance of various genera was found to be representive for most regions of the world. The estimated overall annual yield loss of world's major crops due to damage by plant parasitic nematodes is 12.3 %.

In India, the annual loss inflicted by pests, disease nematodes and weeds is estimated at Rs. 6,000-17,000 crores. Many workers have attempted to assess crop losses caused by plant parasitic nematodes in India. Van Berkum and Seshadri (1970) have calculated these losses in India in terms of money. They estimated the annual losses due to 'ear cockle' disease caused by *Anguina tritici* on wheat, at $10 million, due to *Pratylenchus coffeae* on coffee at $3 million and due to molya disease caused by *Heterodera avenae* in Rajasthan alone at $8 million. Paruthi and Bhati (1981) reported the loss in wheat yield due to *Angunia tritici* ranged from 1-9 %. The yield of okra, tomato, and brinjal suffered 90.9, 46.2 and 2.3% losses, respectively, due to *Meloidogyne incognita* infestation at the rate of 3-4 larvae/g soil under field conditions (Bhati and Jain, 1977). Some important nematode pests are potato cyst nematode, *Globodera*

*rostochiniensis* in the Nilgiries, the citrus nematode, *Tylenchulus semipenetrans* in citrus crops, the burrowing nematode, *Radopholus similis* in banana and the reniform nematode *Rotylenchulus reniformis* in cotton, maize, ginger, millet, cowpea, and blackgram. The loss in wheat yield was up to 42.2 % in the sandy soil of Rajasthan due to *Heterodera avenae* (Mathur *et al.*, 1986).

The natural soil environment harbours a multitude of microorganisms. As many as $10^6$ -$10^8$ bacterial cells, $10^6$-$10^7$ actinomycete cells, $5 \times 10^4$-$10^6$ fungal colony-forming units (CFU) ,$10^5$-$10^6$ protozoa and $10^4$-$5 \times 10^5$ algae were estimated to be present in a gram of field soil taken from the surface (Gottlieb, 1976), while Richards (1976 ) found $1 \times 10^7$ nematode in an area of 1 $m^2$ of fertile soil. Although many of these organism are saprophytic, having little, if any effect on cultivated crops ,the moist soil environment is favourable for the activities of the plant parasitic nematodes and for the growth and multiplication of pathogenic fungi. It is of no surprise, therefore that a variety of interrelationships between these organisms have been demonstrated.

It has long been understood that the development of disease symptoms is not solely determined by the pathogen responsible, but it is dependent on the complex interrelationship between host, pathogen and prevailing environmental conditions. In addition, in nature plants are rarely, if ever, subject to the influence of only one potential pathogen. This is especially true for soil borne pathogens, where there is tremendous scope for interaction with other micro- organisms occupying the same ecological niche. Examples of interactions between soil microbes influencing disease development can be seen in plant parasitic nematodes - pathogen complexes. A disease complex is produced through a synergistic interactions between two organisms. Synergistic interactions can be summarized as being positive where an association between nematode and pathogen results in plant damage exceeding the sum of individual damage by pest and pathogen (1+1>2). Conversely, where an association between nematode and fungus results in plant damage less than that expected from the sum of individual organism, the interactions may be described as antagonistic(1+1<2). Where nematode and fungi are known to interact and are shown to cause plant damage that equates to the sum of individual damage by pest and pathogen, the association may be described as neutral (1+1=2). Although the former two associations can be readily demonstrated experimentally, the

latter can prove difficult to identify, as neutral associations can results in similar plant damage to that seen in additive associations, where nematode and pathogen are known not to interact with one another.

A large number of fungi causing leaf spot diseases and obligate pathogens causing both powdery mildew and downy mildew are known to infect balsam in India. Mostly exhibiting symptoms on leaves and blossom, (Bilgrami *et al.*, 1991), but, those affecting stems or roots are only a few. In India, foot and stem rot of balsam caused by *Fusarium oxysporum* (Bhargava and Singh, 1979). Similarly, *Rhizoctonia solani*, Kuehn has been reported to infect color and root-rot of balsam, in Assam (Roy, 1968) and Rajasthan (Siradhana *et al.*, 1964). Charcol rot of balsam caused by *Macrophomina phaseolina* (Tassi) Goid has been reported only from New Delhi (Kandhari *et al.*, 1979). The literature revealed that the root-rot fungus *Macrophomina phaseolina* which was included in the present study is new report from Madhya Pradesh, India.

*Meloidogyne* spp. (*M. arenaria* Neal and Chitwood, *M. incognita acrita* Chitwood, *M. incognita* (Kofoid and White), Chitwood *M. javanica* Treub and Chitwood and *M. hapla* Chitwood, *Rotylenchuylus reniformis* Linford and Oliveira *Pratylenchus penetrans* (Cobb, Filipjev and Schistaek), *Xiphinema diversicaudatum*, *Aphitenchoides ritzemabosi*, *A. besseyi*, and *A. fragariae* has also been found to be infecting on balsam (Goodey, 1965). In India, *M. arenaria* infecting on balsam is reported from Delhi (Sethi *et al.*, 1964). *Meloidogyne javanica* reported from Kerala, Madras, West Bengal, U.P. (Nadakal and Thomas, 1964; Thirugnanam and Rangaswami ,1967a; Khan, ,2003; Khan *et al.*, 2006) and *M. incognita* reported from Madras and West Bengal (Thirugnanam and Rangaswami, 1967b;Sen and Gupta, 1975). It appeared from these findings that the occurrence of *M. javanica* on balsam from Satna District is a new report from Madhya Pradesh, India.

Frequent and simultaneous occurrence of *Meloidogyne javanica* and *Macrophomina phaseolina* was recorded from several area of Satna District, Madhya Pradesh, where balsam was grown as an ornamental plants. It was observed that there were patches in the flower beds where the balsam plants were badly damaged due to root.-rot as well as stem rot caused by the fungus as compared to near by plants in the

same or different beds. Isolation of nematode from the roots and soil, and fungus from the roots of such plants showed heavy infection of root-knot nematode *M. javanica* and root-rot fungus *M. phaseolina*. Moreover, in other neighbouring areas where these pathogens present singly, the damage was comparatively lesser and there was no sign of stem-rot except the few root showing root-rot symptoms. The fungus was isolated from rotted roots on PDA. The resultant mycelial growth was transferred to the same medium in Petri plates and incubated at 28±2$^0$C. The root-rot pathogen was identified as *Macrophomina phaseolina* (ID No. 5705.03). The culture and specimen were deposited in the Division of Plant Pathology, IARI, New Delhi (ITCC No. 5510). Scanning of literature revealed that root-knot nematode and root-rot fungus constitutes the first disease complex of balsam caused by *Macrophomina phaseolina* + *Meloidogyne javanica.*

Keeping in view the economic importance of balsam as an ornamental plant besides having medicinal values and the association of root-knot nematode and root-rot fungus on the plant, it was considered desirable to study whether this aggravated damage was casual or due to the result of interactive effect of *M. phaseolina* and *M. javanica*. With this aim in view the following aspects have been studied.

(i) Studies on the pathogenicity of root-knot nematodes (*Meloidogyne javanica*) on balsam.

(ii) Studies on the pathogenicity of root-rot fungus (*Macrophomina phaseolina*) on balsam.

(iii) Studies on the effect of individual, concomitant and sequential inoculation of root-knot nematode (*Meloidogyne javanica*) and root-rot fungus (*Macrophomina phaseolina*) on plant growth and disease development.

(iv) Studies on the effect of interactions of different inoculum levels of root-knot nematode (*Meloidogyne javanica*) and root-rot fungus (*Macrophomina phaseolina*) on plant growth and disease development.

(v) Studies on the life-cycle of root-knot nematode (*Meloidogyne javanica*) on balsam in presence and absence of root-rot fungus (*Macrophomina phaseolina*).

(vi) Effect of different dilutions of fungal filtrate of root-rot fungus (*Macrophomina phaseolina*) on the mortality of root-knot nematode (*Meloidogyne javanica*) *in vitro*.

(vii) Effect of different dilutions of fungal filtrate of root-rot fungus (*Macrophomina phaseolina*) on the hatching of root-knot nematode (*Meloidogyne javanica*) *in vitro*.

(viii) Effect of root-extracts of balsam infected with different inoculum levels of root-knot nematode (*Meloidogyne javanica*) on the growth of root-rot fungus (*Macrophomina phaseolina*) *in vitro*.

# *Review of Literature*

# REVIEW OF LITERATURE

Plants develop close association with many soil micro-organisms, especially with fungi and nematodes under field conditions. These organisms independently develop associations with plants that are either beneficial or harmful to the plants. It is, therefore, essential to be aware of the array of organisms influencing the crop and of the nature of the various organismic interactions. One pathogen-one disease concept was the basis of investigations made by many nematologists and plant pathologists. Fawcett (1931) was the first to realize that "nature does not work with pure cultures" and that most plant diseases, particularly root diseases, are influenced by various microorganisms occupying the same habitat. Plant parasitic nematodes and pathogenic root infecting fungi are often regarded as pathogens in their own right, capable of producing a single recognizable disease.

Multiple-parasitic associations and disease complexes are the usual norms under field conditions. The knowledge of nematode-fungal disease complexes has increased greatly. In terms of Atkinson, root gall is a disease caused by minute juvenile worms and the organism of 'Frenching' was associated with the nematodes in producing that disease or rather in making it much more serious (Atkinson, 1892). "Frenching" was an old term for *Fusarium* wilt. Hence, since 19$^{th}$ century scientists were aware of the concept of disease complexes but have failed to understand fully the mechanism of interaction.

Although some research has been conducted in recent years, yet there is great scope of making studies on the complex interactions among plant parasitic nematodes, soil micro-organisms and host roots. The fungus-nematode interactions are numerous and varied that they provide a wide open field for significant research. Weekly parasitic fungal parasites can cause considerable damage once they gain entry into plant roots in the presence of feeding nematodes. The nematodes, therefore, help the saprophytic fungi as invading organisms and increase their pathogenicity. Nematodes provide ready means of entry into the host for the fungus. Undoubtedly, this occurs when root-browsing nematodes cause superficial root injury and so enhance fungal access and enhance secondary pathogenicity of the roots. Literature scanning revealed that several workers have reviewed the work on interaction of phytoparasitic nematodes with fungi on various crops (Powell, 1971; Pitcher, 1976;

Khan *et al.*, 1992; Evans & Haydock, 1993; Chahal & Chahal, 1998; Back *et al.*, 2002).

The frequency of involvement of nematodes and fungi in disease complexes is reflected in the number of crops on which such complexes are recorded and, as the single most destructive nematode species in the world, it is not surprising that *Meloidogyne incognita* has been so frequently reported in disease complexes.

Brodie and Cooper (1964) reported that the mechanical wounding of cotton seedlings failed to increase the susceptibility to either *Rhizoctonia solani* or *Pythium debaryanum* also found that sporangial production of *P. debaryanum* was almost ten times greater in the presence of juice expressed from root-knot galls produced by *M. incognita* than in the presence of juice from healthy roots. These observations indicate that the nematodes made the roots a better environment for fungal development, perhaps most simply by increasing the nutrient supply available. Melendez and Powell (1965) reported galled tissue of both resistant and susceptible varieties of flue-cured tobacco were more favourable sites for penetration and extensive development of fungal hyphae of *F. oxysporum f. nicotianae.*

Batten and Powell *et al.* (1971) observed that if *Meloidogyne incognita* preceded *R. solani* by 10 days or 21 days in roots of greenhouse grown tobacco plants, root-rot was more extensive then when the nematode and fungus were introduced either simultaneously or separately or when *R. solani* was added after artificial wounding. Histological examination of galled roots 72 days after inoculation with *Rhizoctonia solani* revealed extensive fungal colonization in the root-knot susceptible cultivar 'Dixie Bright 101' when *M. incognita* preceded *R. solani* by 21 days. *Rhizoctonia solani* normally nonpathogenic on mature tobacco roots may cause severe losses when present with well established root-knot nematodes infections.

Hazarika *et al.* (1974) studied the interrelationship between *Rhizoctonia solani* and *Meloidogyne incognita* on egg plants (*Solanum melongena* L.) and they showed that the number of galls on roots as well as the number of eggmasses were significantly greater in plants inoculated with nematode and fungus together than in those inoculated with nematode alone. Moreover, the growths of egg plant were not affected significantly by the attack of *M. incognita* or *R. solani* alone or by their combination.

In field studies with okra and tomato, fumigants (ethylene dibromide and methyl bromide, respectively) were applied to field plot to reduce *M. incognita* and *R. solani* and create independent and combined treatments. In untreated plants (plots with both *R. solani* and *M. incognita*), *R. solani* was isolated from the galls of *M. incognita* a week after gall formation. Two weeks later, numerous black sclerotia were found encrusted to the galls. In contrast sclerotia were absent from ungalled regions of the roots. Four weeks after gall formation, substantial root decay occurred. Further more, histological sections revealed that *R. solani* had penetrated cells from the sclerotia attached to the gall surfaces. *Rhizoctonia solani* appeared to have a marked trophic intercellular pattern through the cortex of galled roots toward nematode- induced gaint cells (Golden and Van Gundy, 1975).

Chhabra *et al.* (1977) reported that there was significant reduction in the shoot and root lengths, and their wet and dry weight in the plant receiving the inoculum of a fungus + nematodes simultaneously, nematode + fungus 10 days after, and fungus + nematode 10 days after over control while nematode reduced the shoot length only. Maximum reduction in the plant growth was noticed where fungus + nematodes were inoculated simultaneously. Fungus alone has almost no effect on plant growth reduction.

Goswami and Agarwal (1979) determine the interrelationship between *M. incognita* and *Fusarium oxysporum, F. solani, F. graminearum* and *F. equiseti* on soybean. Seedlings were inoculated with nematode and fungus alone, simultaneously or with one organism preceding the other by 3 weeks. The results showed an antagonistic interaction of *F. oxysporum* and *F. solani with M. incognita*. The fungus when established first reduce symptoms due to the fungus. The interaction of *F. graminearum* and *F. equiseti* with *M. incognita* was synergistic, nematode-infested plants being more severely damaged by the fungi than the non infected plants.

Reddy *et al.* (1979) reported that inoculations of *Phaseolus vulgaris* with *M. incognita* alone or simultaneously with *R. solani* or 10 days prior to fungal infection reduced plant height and fresh weight of shoot and gave the maximum root-knot infection index. Simultaneous inoculations of both the pathogens caused grater damage than either organism acting alone.

A synergistic effect on the root-rotting of okra plants was observed when *M. incognita* and *R. bataticola* were combined together at an inoculum level of 400 larvae and 4 g mycelial mat per 100 cc soil after 48 days of sowing. Biochemical analysis of the okra roots revealed large accumulation of total phenols, proteins and proline in the infected roots with *M. incognita* alone and in combination with *R. bataticola* over healthy roots, while total sugar decreased in infested roots. A pronounced increase in proline was observed in *M. incognita* and *R. bataticola* as compared to *M. incognita* infected roots alone (Sharma *et al.*, 1980).

Kleineke-Borchers (1982) examined the effect of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on the auxin and cytokinin content of tomato plants after single and double infection. *Meloidogyne incognita* and *Fusarium* caused an increase of auxin content in roots and stems. After double infection, the content of IAA further increased which might be responsible for early typical wilt symptoms and cytokinin content was also increased by root-knot nematode, but decreased by *Fusarium.*

There may be more nutrient in root exudates from plants infected by nematodes. Exudates from tomato roots infected by *Meloidogyne incognita* increased the *in vitro* germination rate of *F. oxysporum* microconidia and contained more carbohydrates and reducing sugars, of which the latter had a positive effect on fungal growth (Kleineke-Borchers and Wyss, 1982). Noguera and Smits (1982) found a similar stimulation of the fungus but also found fewer colonies of actinomycetes antagonistic to *F. oxysporum* in the tomato root rhizosphere when the roots were infected by *M. incognita.* Gonzalez (1982) also showed that extracts of *M. incognita* infected tomato roots would stimulate the growth and germination of *F. oxysporum*, and found that these extracts contained more carbohydrates and amino acids than those from healthy roots.

Negron *et al.* (1982) reported interaction between *M. incognita* and *F. oxysporum* f. *coffeae* by inoculating coffeae seedlings with both the pathogens singly and in combination either simultaneously or nematode first followed by fungus. Chlorosis, root necrosis, wilting and dwarfing were significantly greater in plants inoculated with fungus 4 weeks after nematode inoculation. Nogura (1983) also suggested that *M. incognita* infested tomato roots become generally susceptible to invasion by *F. oxysporum.*

Chahal and Chhabra (1984) studied that *M. incognita* and *R. solani* separately as well as in combination significantly reduced the shoot length, shoot weight and root – weight as compared to uninoculated control. The synergistic effect of simultaneous inoculation was apparent from the significant reduction of shoot weight and length and root weight in comparison to either of the pathogens alone. Inoculation of *M. incognita* 3 weeks prior to *R. solani* significantly reduced the shoot weight and length in comparison to inoulation of *R. solani* 3 weeks prior to *M. incognita*. It suggests a predisposition of the seedlings roots by nematode for subsequent damage by *R. solani*.

The maximum number of galls and nematode population was observed with the inoculation of *M. incognita* three weeks prior to *R. solani* and this may be because of longer exposure of roots to nematode invasion than in the treatment where *R. solani* was inoculated 3 weeks prior to *M. incognita* and in this case the number of galls as well as the nematode population was minimum.

Abu El-Amayem *et al.* (1985) observed that the soybean plants were damage by either *M. incognita* and *R. solani* but plant growth was depressed even further when plants were inoculated with both organisms, with the maximum effect occurring when the two were inoculated simultaneously.

Al-Hazmi (1985) observed in a greenhouse experiment that severity of root-rot of bean caused by *Macrophomina phaseolina* increased when *M. incognita* were introduced two weeks before the fungus alone. Nematode infection and reproduction were reduced when fungus was introduced first, inoculation with either pathogen or with both generally reduced root-rot in both the culture significantly and also reduced pod weight. Lanjewer and Shukla (1985) reported that rotting of ginger roots by *P. myriotylum* was equally severe in the presence or absence of *M. incognita* but the presence of fungus decreased nematode reproduction. Moreover, Costa Manso and Huang (1986) found that infection by *M. incognita* seemed to be provide the protection to *Phaseolus vulgaris* against *Rhizoctonia solani*.

Mani and Sethi (1987) studied the effect of combined inocula of *M. incognita* and *F. oxysporum* or *F. solani* on the growth of chickpea which was found to be additive in nature. However, when nematode was established earlier than the two fungi the resultant effect was more than additive. Occurrence of *M. incognita* in

combination with either *F. oxysporum* f. sp. *ciceri* or *F. solani*, not only increased the severity of disease but also shortened the incubation period for disease expression.

Varshney *et al.*, (1987) reported the inoculation of cowpea with *M. incognita* and *R. solani* led to breakdown of resistance to both organisms and the greatest decrease in plant dry weight occurred if the nematode was inoculated two weeks before the fungus. Inoculation of chickpea either with *M. incognita* or *F. oxysporum* f. sp. *ciceri* reduced growth of chickpea and highest reduction was obtained with simultaneous inoculation of both the pathogens followed by the treatment in which nematode inoculation preceded the fungus by ten days (Kumar *et al.*, 1988).

Mishra *et al.* (1988) reported that the combined inoculation of *M. incognita* and *R. bataticola* on white jute plants markedly increased the incidence of root-rot over that found with either organism alone. Sheela (1990) observed that the inoculation with *M. incognita* and *F. oxysporum* on black pepper had a synergistic effect on growth retardation. She observed that the fungal infection in stem portion was more severe in plants with prior nematode inoculation than that in simultaneous inoculation or fungus prior followed by nematode respectively.

The existence of a general, systemic effect on susceptibility to *F. oxysporum* was confirmed by El-Sherif and Elwakil (1991), who used split tomato root systems and inoculated one half with *M. incognita* and the other with the fungus. An increase in susceptibility to fungal invasion was evident in the half of the root system free of nematodes. Thus there is much evidence to indicate that physiological changes caused by nematodes increase plant susceptibility to fungi, but little evidence to suggest the actual mechanisms involved.

Siddiqui and Husain (1991) observed the growth of chickpea and nodulation were adversely affected by the presence of both *M. incognita* race 3 and *M. phaseolina*. Disease severity increased with increasing inocula and various combinations of *M. incognita* and *M. phaseolina* had a synergistic effect on plant growth reduction. The rate of nematode multiplication was density dependent. The multiplication and root galling was increased with the increase in inoculum levels while root rotting increased with the increase in the combined inocula of *M. phaseolina* and *M. incognita*.

Simultaneous inoculation of *M. incognita* and *F. solani* caused greatest reduction in the plant growth followed by the sequential inoculation of nematode 15 days prior to fungus and of fungus 15 days prior to nematode. Presence of *F. solani* with *M. incognita* also showed reduced multiplication rate of the nematode and increased root-rot caused by fungus as compared to the individual inoculation of these pathogens (Khan and Husain, 1991).

Dwivedi *et al.* (1992) indicated that the effect of the *M. incognita* in combination with the *F. oxysporum* enhanced the suppression of growth of plants including bacterial nodules. Of the two organisms, *M. incognita* affected the plant growth characters to a greater extent in comparison to fungus, however, maximum growth reduction was observed when, both the organisms were present at higher level. The bacterial nodulation was adversely affected in the presence of both the organisms. The nematode development and multiplication was also affected by the presence of fungus and maximum gall index was found at higher inoculum levels.

Fazal *et al.* (1994) reported the interaction between *M. incognita* and *Fusarium oxysporum* f. sp. *lentis* on lentil using various combinations of each pathogen. Individually, *F. oxysporum* f. sp. *lentis* was the most aggressive pathogen. At all combinations, reduction in growth parameter in concomitant inoculation was greater than the additive of the pathogens acting independently, thus showing a synergistic relationship. Nematode multiplication and galling in the presence of fungus were significantly reduced.

Swain and Kar (1994) reported the disease complex involving *M. incognita* and *F. pallidoroseum* on black gram. Wilting was maximum in treatments receiving *M. incognita* 7 days before *F. pallidoroseum* followed by simultaneous inoculation of both the pathogens. Multiplication of *M. incognita* was adversely affected by *F. pallidoroseum.*

Rao and Krishnappa (1994) reported that the inoculation of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *ciceri* alone adversely affected the growth of chickpea cv. Annegiri-1. The reduction of plant growth characters and severity of root-knot and wilt incidence were more with increased levels of nematode and fungus inoculum and the interaction effects were more in combination than either of them alone. Inoculation of nematode along with fungus at lower levels resulted in higher

percentages wilt incidence when compared with fungus alone. However, the greatest wilt incidence was observed when inoculated with second stage juveniles of *M. incognita* /g soil and 25 g inoculum of *F. oxysporum* / 500 g soil. Presence of root-knot nematode along with fungus adversely affected the root-galling.

Samathanum and Sethi (1994) reported that the *Rhizoctonia bataticola* was the only organism which affected seedling emergence, yet the presence of the nematode and other saprophagous fungus tended to modify this effect in a differential manner, exhibiting neutral relationship with *Penicillum chrysogenum* and *Trichoderma viride* slightly antagonistic with *M. incognita* or synergistic interaction with *Alternaria alternata.* In addition, *A. alternata* wherever added tended to produce abnormal seedling having curly shoot, failure in emergence of plumule, or bloating of stem.

France and Abawi (1995) studied the interaction between *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *phaseoli* (Fop) on selected bean genotypes. Four bean genotypes (IPA-1, A-221, A-107 and Calima), representing all possible combinations of resistance and susceptibility to *F. oxysporum* f. sp. *phaseoli* and *M. incognita*, were each inoculated with three population densities of these pathogens. In *F. oxysporum* f. sp. *phaseoli* susceptible lines (IPA-1 and A-211), the presence of *M. incognita* contributed to an earlier onset and increased severity of Fusarium wilt symptoms and plant stunting. However, the *Fusarium oxysporum* f. sp. *phaseoli* resistant Calima developed symptoms of Genotype A-107 resistant to both (*M. incognita* and *F. oxysporum* f. sp. *phaseoli*) and exhibited Fusarium wilt symptoms and plants stunting. Root galling and reproduction of *M. incognita* were increased on the *M. incognita* susceptible cultivars. However, these factors were decreased as the inoculum density of *F. oxysporum* f. sp. *phaseoli* was increased. It was concluded that severe infection of bean roots by *M. incognita* increased the severity of Fusarium wilt on *F. oxysporum* f. sp. *phaseoli* susceptible genotype and may modify the resistant reaction to *F. oxysporum* f. sp. *phaseoli.*

Anwar *et al.* (1997) studied the interaction between *Meloidogyne incognita* and *Rhizoctonia solani* on soybean and observed significant alterations in chlorophyll a and b by simultaneous, sequential and individual inoculations but more damage occurred with simultaneous inoculation than other treatments.

The incidence of the rhizome-rot of ginger was higher when *M. incognita* and *F. oxysporum* were present concomitantly than either of the two organism alone. Vascular browning was observed in case of fungus alone and nematode + Fungus. However, no such browning was recorded in nematode alone and control. From this study, it could be inferred that the disease incidence aggravated when the nematode and fungus interact than either of the pathogen alone Makhnotra *et al.* (1997).

Effect of rhizosphere fungi and root-knot nematode on mungbean *Vigna radiata* variety ML-131 under the pot culture conditions was reported by Chahal *et al.* (1997). Simultaneous infection of *M. incognita* and pathogenic fungi (*Fusarium oxysporum* and *Macrophomina phaseolina*) caused more damage to the plants than individually.

Variability among *F. oxysporum* f. sp. *lycopersici* isolates in their ability to interact with *Meloidogyne incognita* race-1 was observed by Suleman *et al.* (1997). Isolates of *F. oxysporum* f. sp. *lycopersici* obtained from different populations of the fungus varied in their ability to form a disease complex with the root-knot nematode. All race-1 isolates of *Fusarium oxysporum* f. sp. *lycopersici* were similar in being unable to overcome monogenic resistance in tomato during co-infection with nematodes. Isolates were also similar in causing a greater severity of wilt and vascular discolouration during co-infection of tomato with nematodes. Individual isolates differed with regard to the increase in wilt symptoms severity and extent of vascular discolouration induced during co-infection, suggesting possible heterogeneity among *F. oxysporum* populations in the degree of interaction with *M. incognita.*

Charu and Trivedi (1998) reported the effect of interaction between *Meloidogyne incognita, Fusarium oxysporum* and *Rhizoctonia bataticola* on chickpea. A combination of root-knot nematode, *Meloidogyne incognita* with *F. oxysporum, R. bataticola* and *Macrophomina phaseolina* caused more severe disease and yield losses in chickpeas. All the three pathogens were inoculated in different combinations to assess their role in disease severity. Reduction in plant growth, severity of root-knot and wilt incidence were greater in the combined treatments as compared to the pathogens alone. The reduction in shoot, root length and weight was more pronounced when *F. oxysporum , Macrophomina phaseolina, R. bataticola* and

*Meloidogyne incognita* were combined together followed by each fungus with nematode as compared to control and the each fungus alone.

Bhagwati *et al.* (2000) reported that either *M. incognita* or *F. oxysporum* f. sp. *lycopersici* significantly reduced the plant vigour compared to uninoculated check. However, synergistic effect of the pathogens (N+F) or where nematode inoculation preceded 10 days to fungus (N< F10). The possible explanation for comparatively greater damage in these two treatments (F< N10) may be attributed to the prior invasion of nematode into the roots their by making the host more favourable for fungal infection by offering a metabolically rich substrate, and/or nematode might also modify the rhizosphere there by favouring the fungal growth. In N+F and N< F10, an early expression of wilt symptom by 20 days was also observed as against to such symptoms in F< N10 at the same time of observation. The intensity of wilt (wilt indices) after 40 and 60 days of inoculation was significantly higher in N< F10 and N+F when compared with F < N10 and F treatments. The number of galls and egg masses and nematode population in soil was significantly reduced in N+F and N< F10. This may be due to reduced root system in these treatments which was unable to support large number of galls, consequently further reproduction of the nematode was arrested.

Patel *et al.* (2000) observed interaction of *Fusarium oxysporum* f. sp. *ciceri* with *M. incognita* on chickpea cv. Dahod yellow revealed that the organisms either individually or in combination reduced plant height, fresh root and shoot weight significantly but, the reduction was more by nematode as compared to fungus. Among combined inoculations, simultaneous inoculation of both the pathogens had maximum suppressive effect on growth of chickpea plants as compared to preceding or succeeding inoculations, of fungus and nematodes. Root galling and nematode multiplication on chickpea were maximum when nematodes were inoculated alone but it was reduced in the presence of fungus. The fungus alone was able to produce wilt disease but the incubation period for disease development was reduced and severity of the disease increased when root-knot nematode was present with fungus. Maximum wilting of plant was observed when the fungus and nematodes were inoculated simultaneously.

Reddy *et al.*, (2001) observed that nematode alone significantly increased pre-emergence damping-off incidence from 37.6 to 43.8% with the increased in inoculum levels of either nematode (*M. incognita* ) or fungus (*P. aphanedermatum* ).Moreover, nematode along with fungus increased the pre-emergence damping-off from 40.0% to 47.0% in addition, the post-emergence damping-off increased from 40-50% when both the organisms were present.

Singh and Goswami (2001) reported *Meloidogyne incognita* enhanced wilting of cowpea cultivar Pusa Komal when inoculated in combination with *Fusarium oxysporum*. Nematode inoculation preceded by fungal inoculation showed maximum effect followed by the treatment where both the pathogen were inoculated simultaneously. Presence of nematodes not only predisposed the host but also shortened the incubation period disease expression. Both the pathogens interacting simultaneously or nematode infecting prior to fungus, affect nodulation.

Pathak *et al.* (2004) indicated that wilt appeared only in those treatments where *F. oxysporum* was inoculated either singly or in combination with root-knot nematodes. Inoculation of only root-knot nematode did not produce wilting in cauliflower plants. Maximum wilt score and wilt symptom index was observed in plants, which received nematodes 14 days before the inoculation of wilt fungus, followed by nematode 7 days before fungus. In general, there was more wilting in cauliflower plants when nematode was inoculated prior to fungus. In the present findings high wilting has been recorded in plants inoculated with both *M. incognita* and *F. oxysporum*. Although wilt severity was higher when nematode was inoculated prior to fungus and highest when nematode was present 14 days prior to fungus in cauliflower. The reduction in number of root-galls and decrease in soil nematode population in treatments where fungus was present along with nematode, suggests that *F. oxysporum* was inhibitory to nematode multiplication. The plant exposed to *F. oxysporum* without nematode recording low wilt disease severity than the plant where nematode was present together with fungus. The low disease severity in such plants suggest the delay in the entry of pathogen due to absence of predisposing agent (*M. incognita*). Presence of both pathogens had more deleterious effect on cauliflower plants than with either

pathogen alone, which indicated a positive interaction between them in cauliflower.

On sugar-cane, rotting of the root system was increased when both *Curvularia lunata* and *Meloidogyne javanica* were present with greatest damage occurring when they were inoculated together but with significant damage even when the inoculations were ten days apart (Khurana and Singh, 1971).

Goswami *et al.*(1976) reported the highest percentage of wilting of tomato plants when *M. javanica* was inoculated three weeks before infection with *Rhizoctonia bataticola* (Taub.) Briton Jones. Moreover, the percentage of wilting was less after simultaneous inoculation of pathogens, fungus infection alone or fungus infection three weeks before nematode inoculation.

Nath *et al.* (1984) observed that root-knot nematode increased the extent of damage by pre and post emergence phases of damping-off caused initially by any of the three test fungi. Association of *Fusarium* + *Rhizoctonia* with or without nematode produced low percentage and long duration of damping-off, low root-knot index and low recovery of nematode population. Nematode + *Rhizoctonia*, in addition to causing high percentage of damping-off and root-knot index, also affect maximum reduction in shoot length and high nematode count of the soil. Mechanical wounding of roots, devoid of nematode, in the presence of any of the damping-off fungi exhibited low percentage of damping-off, minimum reduction in plant height and their fresh weight in comparison to the treatments, whose nematode replaced mechanical wounding.

Inoculation of 3 days old seedlings of chickpea with 100 eggs of *M. javanica* per kg soil or 1 ml suspension of mycelia and conidia of *F. oxysporum* f. sp. *ciceri* (Padw.) Matuo and Sato. In this experiment there was reduction in plant height, 1.18% with fungus alone, 17.53% with nematode alone and 19.47% with nematode and fungus together after 99 days. Reduction in fresh weight of root was 3.78%, 30.61% and 49.3% respectively. Combined infection caused the maximum damage to the plants (Sharma, 1985)

Goyal and Gupta (1986) inoculated chickpea seedlings with *Meloidogyne javanica* and *Rhizoctonia bataticola* simultaneously or alone. The combined

inoculations irrespective of time reduced growth parameters compared with individual inoculation. Number of galls was significantly reduced when nematodes were inoculated 7 days before the fungus.

Sakhuja *et al.* (1986) observed that the multiplication of *M. javanica* and the galling on groundnut was adversely affected by *R. bataticola* and *Fusarium solani.* Reduction in multiplication was maximum wherever one or both the fungi were inoculated simultaneously with the nematode. Introduction *of F. solani* one week after the nematode did not reduce galling and the nematode population in soil and roots significantly. *Rhizoctonia bataticola* proved more antagonistic to *M. javanica* than *F. solani.*

Upadhyay and Dwivedi (1987) found highest wilt symptoms in chickpea plants inoculated simultaneously with both *M. javanica* and *F. oxysporum* f. sp. *ciceri,* followed by inoculation of the nematode preceding the fungus and fungus preceding the nematode. The maximum number of root galls were recorded on roots inoculated with nematode alone and minimum number on roots where inoculation of the fungus preceded the nematode. The maximum reduction in shoot weight occurred where inoculation of nematode preceded that of the fungus.

Kanwar *et al.* (1988) observed that the plant growth was significantly reduced when nematode (*M. javanica*) was inoculated 3 weeks prior to fungus (*R. solani*) which may be due to the resultant fungal disease severity. However, loamy sand favoured the plant growth wherein the buildup to nematode population was also more.

Generally, plant growth was reduced when nematode inoculation was done 3 weeks prior to the fungus. The number of bacterial nodules was minimum in nematode alone treatment. As far as the soil type was concerned, loamy sand appeared better for plant growth than the other types used. The number of bacterial nodules was also reduced significantly in presence of both the pathogens with maximum reduction in nematode alone treatment.

The number of galls was significantly decreased in the presence of fungus. It was minimum when nematode and fungus were inoculated together compared with nematode alone.

*Macrophomina phaseolina* interacted with *M. javanica* on lentil to cause most damage when the two organisms were inoculated simultaneously but the fungus inhibited nematode reproduction, especially when it was inoculated first (Tyagi *et al.*, 1988).

Gupta *et al.* (1989) reported that the number of galls were reduced when nematode and fungi were added simultaneously. Moreover, the highest reduction in plant growth parameters was observed when *R. bataticola* was added with nematode as compared to *R. solani.*

Mehta *et al.* (1989) reported that the reduction in plant growth characters of watermelon was more discernible when nematode *M. javanica* was inoculated higher to the *R. solani* at both the levels of inoculum (500 and 1000) as compared to these individual inoculation.

Khan and Hosseni (1991) observed synergistic interaction between *M. javanica* and *F. oxysporum* f. sp. *ciceri* on chickpea cultivars both in concomitant and sequential inoculations wilt symptoms were most prominent in the presence of *M. javanica* in concomitant inoculation than in sequential ones. Resistance of Pusa-212 recorded with the inoculation of fungus alone, was broken in the presence of nematode.

Anwar *et al.* (1993) observed chickpea seedlings were inoculated with *M. javanica* and *R. solani* alone or simultaneously or with *M. javanica* one week before or one week after *R. solani* inoculation. Greatest reduction in shoot and root length and fresh and dry weight occurred with simultaneous inoculation of both pathogens. This was followed by nematode inoculation one week before and one week after the fungus.

Shah *et al.* (1994) *Capsicum annuum* seedlings were inoculated in a pot experiment with *M. javanica, Aspergillus niger* and *R. solani* alone or in various combinations. These pathogens reduced growth parameters significantly when inoculated alone. Maximum reduction occurring with *R. solani* and least with *A. niger* reduction in growth parameters were further increased with concomitant inoculation of *R. solani and M. javanica.*

Fazal *et al.* (1994) studied the effect of individual and combined inoculations of *Meloidogyne javanica, Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *ciceri* on chickpea in various combinations. Singly *F. oxysporum* f. sp. *ciceri* was the most aggressive pathogen. In concomitant inoculations, reduction in growth parameters was synergistically than caused by either of the pathogens alone. All the three pathogens adversely affected nodulation singly as well as in concomitant inoculations, and reduction in nodulation followed a similar trend in plant growth. Rate of nematode multiplication and galling was significantly reduced in the presence of both the fungi.

Griffin and Thyr (1978) observed that inoculation of 14 days old seedlings with *M. hapla* followed by *F. oxysporum* after 30 days, there was significant reduction in the growth of plants susceptible variety and not of the resistant variety.

The severity of black root-rot of peanut caused by *Cylindrocladium crotalariae* was enhanced by the presence of *M. hapla* even on *Cylindrocladium* black rot-resistant roots (Diomande and Beute, 1981a), but *Macroposthonia ornata* was less effective at predisposing peanut roots to infection by this fungus (Diomande and Beute, 1981b).

Khan and Muller (1982) studied the interaction between *R. solani* and *M. hapla* on radish plants. They, observed that prior infection of roots by nematodes favoured the colonizing capability of the fungus. Galls were preferred by the fungus and mycelium accumulated over them. Vigorous mycelial growth and abundant sclerotial formation was observed on galls. Non-galled portion did not show sclerotial formation, but in contrast more hyphal growth and penetration was observed in the roots inoculated with the fungus alone. They further suggested that apparently the physiological changes, especially, in the galled regions due to nematode infection, predisposed the roots for invasion and rapid colonization by the fungus. *Meloidogyne hapla* appeared to exacerbate the loss of alfalfa seedlings in soil contaminated with *Pythium ultimum* (Townshend, 1984).

Griffin and Thyr (1986) observed that *Fusarium* wilt of lucerne synergistically increased from combined inoculations of *M. hapla* and *F. oxysporum* on root-knot susceptible var. Ranger but not on resistant var. Nev Syn xx variety. There was positive correlation between plant growth suppression and incidence of vascular

bundle infection and discolorations. Maximum reductions resulted from sequential inoculations of two pathogens.

Root-rot of alfalfa caused by *Phytopthora megasperma* was also made more severe by *M. hapla* especially when inoculation of the nematode preceded that of the fungus (Griffin *et al.* 1988).

Gracia and Mitchell (1975a) studied the interactions by exposing groundnut seedlings in autoclaved soil to predetermined inoculum densities of the pathogens alone or in combinations. No single pathogens (*Phythium myriotylum, F. solani, R. solani* and *M. arenaria*) caused significant damping-off at the density employed. *P. myriotylum* interacted significantly with *F. solani* and *M. arenaria* but not with *R. solani* in causing damping-off.

Gracia and Mitchell (1975b) reported that *M. arenaria* interacted synergistically with *Phythium myriotylum* on peanut causing severity in pod rot and also interact with *Aspergillus flavus* link to cause increased root disease which was accompanied by reduced nematodes multiplication (Patel *et al.* 1986).

Root-knot nematodes break the resistance of crop plants against soil borne pathogens. This aspect has been reviewed recently by Mai and Abawi (1987), Hasan (1993) and Prot (1993). Perhaps *Meloidogyne* modifies the resistance of the host to other parasites. Conversely, the plugging of xylem vessels by a wilt fungus prevents nutrient and water uptake and so decrease host vigour apparently makes them more vulnerable to challenges by other pathogens such as *Meloidogyne*.

Harrison and Young (1940) observed that root-knot nematode reduced the wilt resistance of several varieties of tomato in glass house experiments. Twenty tomatoes lines and varieties inoculated with wilt fungus and root-knot nematode, more found to be susceptible including those resistance to *Fusarium oxysporum* f. sp. *lycopersici.*

The bridging and grafting experiment on tomato revealed that a factor emanating from the *Meloidogyne incognita* and tomato interaction was distally translocated across a resistant scion to the upper foliage and seemed to transfer a host plant genetically resistant to *F. oxysporum* f. sp. *lycopersici* into one that was susceptible cultivars to become even better hosts following the nematode-host interaction (Sidhu and Webster, 1978).

Noguera (1980) observed that the germination and growth of *F. oxysporum* in root extracts of a *Fusarium* resistant tomato was significantly greater when the tomatoes had been infected with *M. incognita* and was more marked with the increasing length of time that the plants had been infected. After four weeks of nematode infection, the amount of amino acids in the roots had increased by 50 percent and the carbohydrates by 112 percent. It is attributed to the reason for the breaking of resistance to *Fusarium.*

Price (1980) studied the interaction of *V. dahliae, F. oxysporum* f. sp. *lycopersici* race-1, *M. javanica, M. incognita* and *M. hapla* on four hybrid tomato cultivars bred for resistance to all the pathogens. Galling due to *M. hapla* was decreased in the presence of *F. oxysporum* f. sp. *lycopersici.* Infection by *F. orysporum* f. sp. *lycopersici* significantly increased in the presence of *V. dahliae* and *Meloidogyne* sp. Hadiastono (1981) found that inoculation of *Meloidogyne* sp. resulted in tomato varieties originally resistant to *F. oxysporum* becoming susceptible, *Fusarium* depressed the development of *Meloidogyne* sp.

Salem (1982) inoculated simultaneously wilt susceptible and resistant cotton cultivars with *M. incognita* and *F. oxysporum* and found increase in the wilt symptoms particularly in the resistant cultivars. The presence of *Fusarium* negatively affected the reproduction of *M. incognita* and gall formation. Kleineke Borchers and Urs (1982) investigated changes in susceptibility of tomato plants to *F. oxysporum* f. sp. *lycopersici* after infection by *M. incognita.* They found that *M. incognita* enhanced disease severity due to *F. oxysporum* in susceptible or resistant tomato plants. Quantitative analysis of mycelial growth using glucosamine determination clearly demonstrated that nematode provided better mycelial growth conditions in roots. Exudates of *Meloidogyne* infected roots increased *in vitro* germination rate of conidia and the content of carbohydrates and reducing sugars. These substances and total amount of free amino acids increased within galled roots.

Hasan (1985) investigated the role of *R. solani* and *Phythium aphanidermatum* Edson Fitzpatrick in the breaking down of resistance to *M. incognita* in *Capsicum annuum* cvs. Jawala and Longthin Faizabadi respectively resistant and moderately resistant to this nematode when either of the fungi were inoculated simultaneously or later than the nematodes, there was a breakdown of resistance of Jawala and Longthin

Faizabadi to the nematode. Hasan and Khan (1985) studied the effect of *R. solani*, *Sclerotium rolfsii* and *V. dahliae* in reducing the resistance to *M. incognita* in case of tomato cvs. F-24 C8 (immune) Heinz-1409 (resistant) and T4 (moderately resistant). They found that *V. dahliae* reduced the resistance of all the three cultivars. Egg production was the highest where seedlings were inoculated with the fungi two weeks prior to nematode infection.

Mousa and Hague (1988) studied the influence of the root-knot nematode, *Meloidogyne incognita* on the development of *F. oxysporum* f. sp. *glycines* in wilt resistant and wilt susceptible soybean cultivars. Exposure of Amsay (wilt susceptible) and Coll (wilt resistant) soybean to *Fusarium oxysporum* f. sp. *glycines* in the presence of the nematode resulted in a large increase in the amount of the fungus found in the vascular tissues of the roots and stems as compared to the amount found in plants exposed to the fungus alone. The fungus was isolated from leaves and seeds of the wilt resistant cultivar only when the nematode was present. Mouso and Hague (1989) observed that fungus *Fusarium oxysporum* f. sp. *glycines* play an important role on the invasion and development of *Meloidogyne incognita* on soybean. When *M. incognita* and wilt fungus *F. oxysporum* f. sp. *glycines* were inoculated simultaneously on growing seedlings of the soybean cultivars Ware and Coll, nematode invasion of the root was not affected but gaint cells were invaded by the fungus and destroyed. The over all effect was to reduce the number of females of *M. incognita* and increase the proportion of males

Khan *et al.* (1992) reported that the papaya cvs. Ceylon,C.S.C.Dwarf-1 Mammoth, Ranchi and Washington were resistant to *F. solani*, but became susceptible to it in the presence of *M. incognita*. They further, observed that the association of nematode and fungus on the cvs. Poona Round, Phillipines, Coorg Honey Dew, Singapore and Improved sunrise not only increased the root-rot and plant growth reduction, but also caused early killing of the plants.

Hilocks and Marley (1995) observed the systemic effect of root-knot nematodes on mechanism of resistance of *Fusarium* wilt diseases. Root-knot nematode (*Meloidogyne* spp.) induced profound changes in the structure and function of the xylem tissues incidence of *Fusarium* wilt in number of crops. One or more mechanism may operate to increase the plants susceptibility to infection, depending

upon the host and nematode species involved. Systemic effects on host susceptibility to *Fusarium* wilt diseases have been indirectly demonstrated for some crops, although in most cases the mechanism of the effect is unknown. For cotton and pigeon-pea, a partial breakdown in resistance occur in the presence of root-knot nematode and this is attributed to nematode induced decreases in the effectiveness of vascular occlusion in cotton and to a related phytoalexin response in pigeon-pea.

Rao and Krishnappa (1996) investigated the interaction of *F. oxysporum* f. sp. *ciceri* with *M. incognita* on chickpea cultivar Annegiri in two soil types viz. alfisol and vertisol. The results indicated that the inoculation of fungus either with nematodes or seven days before or after nematode inoculation resulted in significant reduction in fresh weight and dry weight of roots as well as an increase in wilt incidence as compared to the plants inoculated with the fungus alone. Nematode multiplication and root-knot incidence were higher in the alfisol, however, on the other hand reported that the fungus growth and wilt incidence was higher in vertisol soil.

Rao and Krishnappa (1997) conducted a survey on prevalence of *Meloidogyne-Fusarium* wilt disease complex of chickpea in Karnataka they also observed effect of the interaction of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *ciceri* on root-knot disease and wilt incidence in chickpea cultivars susceptible to *M. incognita*. Root-knot index was lowers in the presence of *F. oxysporum* f. sp. *ciceri*. There was an increase in wilt incidence in the presence of nematode and 2 wilt resistant cultivars B Dala-3 of nematode and Acroclhi lost their resistance in the presence of *M. incognita*.

Many papers discuss the actual interactions between nematodes and root-rot fungi, however, not all reports demonstrate the interactions. Jackson and Minton (1968) investigated the invasion of peanut pods by *Aspergillus flavus* in the presence of *Pratylenchus brachyurus* to establish if there was a relationship between the two organisms in field microplots. In trials from 1965 to 1967, while number of *A. flavus* and *A. niger* in the kernels were unaffected by the presence of lesion nematodes, total numbers of all fungi in kernels at pod maturity were increased. The authors concluded that the results showed no interaction between *P. brachyrus* and *A. flalvus*. Abawi and Barker (1984) researched the effects of tomato cultivar, soil temperature and

levels of *Meloidogyne incognita* on root necrosis and *Fusarium* wilt. Factorial analysis of data from glasshouse pot trials showed no interaction between low levels of the nematode and *F. oxysporum* irrespective of the resistance status of the tomato plant to the nematode.

Culture filtrates taken from cultures of different fungi have been used by many workers to show that fungi produce metabolites which prevent hatching of and even kill nematode juveniles. Shukla and Swarup (1971) studied a pathogenic strain of *Sclerotium rolfsi* was frequently associated with tomato plants infested with *Meloidogyne incognita*. The fungus filtrate from a 10 days old culture inhibited larval hatch up to N/8 concentration and was lethal to the larvae up to N/4 concentration. Shoot weight of tomato increased significantly with the addition of 100 ml fungus filtrate to nematode-infested soil. It is suggested that the lethal effect of the fungus filtrate on the nematode is not only due to low pH but also due to some inhibitory substance present in the filtrate.

Alam *et al.* (1973) studied the effect of culture filtrates of *Helminthosporium nodulosum, Trichoderma lignorum, Curvularia tuberculata, Penicillium corylophilum*, and *Aspergillus niger* on the mortality of *Hoplolaimus indicus, Tylenchorhynchus brassicae*, larvae of *Meloidogyne incognita* and the larval hatch of *M. incognita*. They found that the culture filtrates of all the fungi were toxic to tested nematodes.

Chhabra *et al.* (1978) observed the effect of different concentrations of culture filtrate of *Fusarium moniliforme* on hatching and larval mortality of *M. incognita*. They reported the maximum inhibition of hatching and larval mortality in undiluted filtrate and further found that the egg masses in undiluted filtrate were completely blackened. Haseeb and Alam (1984) measured the reproduction of six different species of plant-parasitic nematodes on tomato plants grown in soil treated with *R. solani* culture filtrate and found that the reproduction of all of them was decreased.

Khan *et al.* (1984a) reported that the mortality of root-knot nematode in the culture filtrates differed with the species of *Aspergillus, A. niger*, A. *candidus, A. flavus* and *A. fumigatus* were more toxic than other species and mortality was directly proportional to the concentration of filtrates and the duration of exposure. The different dilutions of fungal filtrates also significantly inhibited hatching of *M.*

*incognita*. Larval emergence was, however, inversely proportional to filtrate concentration. It was, significantly low up to S/100 but there was no hatching in the 'S' concentration of *A niger, A candidus, A flavus and A fumigatus* Khan *et al* (1984b) reported that the culture filtrates of *Aspergillus niger* and *Rhizoctonia solani* moderately improved plant growth, reduced larval penetration, suppressed nematode reproduction and gall formation on tomato root. Culture filtrate of *A niger* was distinctly more effective than *R solani*. Dahiya and Singh (1985) showed that *Aspergillus niger* culture filtrate killed juveniles of Meloidogyne spp. and also interfered with hatching. Vaishnav *et al*, (1985) also used an activity assay to show that culture filtrates of *Aspergillus* spp. would affect the activity of *M arenaria*

Sharma and Saxena (1992) showed that the culture filtrate of *Rhizoctonia solani* and *Trichoderma viridae* adversely affected the hatching of larvae *of M incognita* with the latter more adversely influenced. The culture filtrates of the two fungi when mixed together did not moderately influence the relative toxicity of the culture filtrates of the two fungi separately. Samathanum and Sethi (1994) observed that the increase in concentration of culture filtrate of the tested fungi (*Rhizoctonia bataticola, Alternaria alternata, Aspergillus flavus, Penicillium chrysogenum, Trichoderma viride*) and period of exposure induced greater inhibition of the hatch as well as mobility of second stage juveniles of *M. incognita*. Culture filtrate of *T viride* at lower concentration and shorter period of exposure resulted in enhanced hatch. Amongst the fungi studied, culture filtrate of *R. bataticola* seems to be more potent in inhibiting of *M. incognita*.

Ashraf and Khan (2005) reported that the inhibition of the hatching and rate of mortality of *M. javanica* increased in the culture filtrate of *P. lilacinus* as compared to other tested fungi viz., *Talaromyces flavus*, *Gliocadium virens* and *Cladosporium oxysporum*. However, *Talaromyces flavus* showed least effect against *M. javanica*, the S/100 concentration of *T flavus* was slightly hatch stimulatory. Percentage mortality and hatching of *M javanica* was directly proportional to the concentration of fungal filtrates and exposure time

Pandey *et al* (2006) observed that the culture filtrates of seven isolates of *P lilacinus* and two isolates of *Trichoderma harzianum* were nematicidal to the second stage juveniles ($J_2$) of *Meloidogyne incognita* producing galls on the betelvine (*Piper*

*betle* L.) roots. The percentage of juvenile mortality increased with the exposure time at all concentrations of the culture filtrates, even at 100 times dilution. The isolates of *P. lilacinus* and *T. harzianum* were also found to have significant parasitic potential on *M. incognita* eggs leading to non emergence of juveniles from egg masses. It was also recorded that only the reproductive phase of the fungi was responsible for this ovcidal action.

Investigating the epidemiology of maize root-rot in South Africa, Chambers (1987) isolated nematodes and fungi from naturally infected field plots. *Helminthosporium pedicellatum, Fusarium moniliforme* and *Pratylenchus* spp. were the most commonly isolated organisms. The numbers of root-lesion nematodes were not significantly correlated with fungus frequency or root-rot development. It was suspected that an interaction had occurred but due to the small nematode infestation the effect could not be quantified.

Doyle *et al.* (1987) described the results of six field experiments started in 1978 to investigate the cause of wheat yield decline in New South Wales, Australia. Early experiments showed the presence of *Pratylenchus thornei* in soils where yield decline was a problem. Application of aldicarb improved yield (32-78%) by reducing nematode populations but had no effect on root-rot. Fumigation with methyl bromide had greater effects on yield than aldicarb because it controlled both nematodes and common root-rot, whereas aldicarb oniy controlled nematodes. Nematodes and root-rot fungi were both implicated in wheat yield decline but interactions were not demonstrated.

In glasshouse trials, Abawi and Jacobsen (1984) showed that there were no interactions between *Heterodera glycines, Fusarium* and *Phythium* spp. on kidney bean at a range of *H. glycines*, population densities from 1 to 100 eggs $cm^{-3}$ of soil. It is possible that the number of case in which no interactions are reported may under estimate the frequency with which they are observed, as such results may be considered less exciting and less worthy of publication.

# *Materials and Methods*

# MATERIALS AND METHODS

The most widely occurring and economically important pathogens viz., root-knot nematode (*Meloidogyne javanica*) and root-rot fungus (*Macrophomina phaseolina*), and an important ornamental host plant, balsam (*Impatiens balsamina*) were selected for the present study.

## PREPARATION AND STERILIZATION OF SOIL MIXTURE:

The soil collected from a fallow field of A.M.U. campus was sieved through 16 mesh sieve and mixed with sieved river sand and organic manure in the ratio of 3:1:1, respectively. Throughout the course of studies, 6 inch pots were filled with this soil mixture @ 1 kg/pot. A little amount of water was poured in each pot to just wet the soil before transferring to an autoclave for sterilization at 20 lb pressure for 20 minutes. Sterilized pots were allowed to cool down at room temperature before use for experiments.

## RAISING AND MAINTENANCE OF TEST PLANTS:

Seeds of *Impatiens balsamina* were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 2 minutes and washed thrice in sterilized distilled water. The seeds were then sown in 12 inch earthen pots for raising the seedlings. Two weeks old well established and healthy seedlings were used for experimental work throughout the course of investigation.

## RAISING AND PREPARATION OF NEMATODE INOCULUM:

The pure culture of *Meloidogyne javanica* was raised on brinjal plant, using a single eggmass collected from infected plant of balsam. The eggmass was surface sterilized by treating it with 1: 500 aqueous solution of chlorox (Calcium hypochloride) for 5 minutes. Treated eggmass was washed thrice in distilled water. The eggs in the eggmass were allowed to hatch out at 28 ± 2 °C under aseptic conditions on a sieve layered with tissue paper and kept in a Petridish containing sufficient amount of sterilized distilled water. Seedling of brinjal grown in 12 inch clay pot, containing sterilized soil was inoculated with the second stage juveniles so obtained. Nematodes were extracted from the soil after two months, according to Cobb's shifting and gravity methods followed by Baermann funnel technique (Southey, 1970). Nematodes so

obtained were used for inoculating fresh brinjal seedlings growing in 12" clay pots containing sterilized soil. Second stage larvae of root-knot nematode infected the roots and multiplied.

After 2-3 months, the eggmasses from heavily infected roots of brinjal on which pure culture of *Meloidogyne javanica* multiplied were handpicked with the help of sterilized forcep. These eggmasses after being washed in distilled water were placed in sieve with a layer of double tissue paper. The sieve was placed over Petridish (10 cm in diameter) containing water. The water level was kept in such a way that it just touched the lower portion of the sieve having eggmasses. A series of such assemblies were kept to obtain large number of second stage juveniles required for inoculations. After every 24 hours the hatched out larvae were collected along with water from the Petridish in a beaker and fresh water added to the Petridishes.

The water suspension of root-knot nematode was thoroughly stirred for making homogenous distribution of nematodes before taking 2 ml of suspension in the counting dish for counting the number of nematodes under the stereoscopic microscope. An average of five counts were taken to determine the density of nematodes in the suspension. Volume of water in the nematode suspension was so adjusted that each ml contained about 100 nematodes, which was done by either adding more water or decanting the excess amount of water so that 10 ml of this suspension contained 1000 second stage juveniles of root-knot nematode.

**ISOLATION OF TEST FUNGUS FROM ROOTS OF BALSAM:**

Balsam plants showing distinct root galls and exhibiting root-rot and stem rot symptoms were collected in polythene bags. Serial washing technique was employed to isolate fungus from infected roots. Roots were transferred to a sterilized dish containing sterilized distilled water and gently freed of soil particles. The process was repeated till all the soil particles were removed. The roots were cut into small pieces approximately 5mm and transferred to Petridishes containing 0.1% mercuric chloride solution. After one minute, root pieces were washed at least three times in distilled water and dried on filter paper. Five of these root pieces were then placed in each Petridishes containing potato-dextrose agar (PDA) having following composition:

| | |
|---|---|
| Peeled potato | 200 g |
| Dextrose | 20 g |
| Agar - Agar | 20g |
| Distilled Water | 1000 ml |

Inoculated Petridishes were incubated at 28 ± 2 °C for 10 days. The fungi which developed on root segments were examined and identified. On confirmation of its identity as *Macrophomina phaseolina*, its pure culture was prepared.

**RAISING AND MAINTENANCE OF FUNGUS CULTURE**

The isolated fungus *Macrophomina phaseolina* was further raised on Richard's liquid medium having the following composition:

| | |
|---|---|
| Potassium nitrate | - 100.00 g |
| Potassium dihydrogen phosphate | - 5.00 g |
| Magnesium sulphate | - 2.50 g |
| Ferric chloride | - 0.02 g |
| Sucrose | - 50.00 g |
| Distilled Water | - 1000 ml |

The medium was prepared and sterilized in an autoclave at 15 lb pressure for 15 minutes in 250 ml Erlenmeyer flasks, each containing about 80 ml of Richard's medium. Small bits of the fungal mycelium *M. phaseolina* were transferred to these conical flasks. Inoculated flasks were incubated at 28±2°C for about 15 days to allow growth of the fungus. Pure culture of *M. phaseolina* was continuously maintained on PDA contained in the test tubes by re-inoculation of the fungus every after one month.

**PREPARATION OF FUNGAL INOCULUM:**

After incubating the conical flasks for about 15 days, the liquid medium was filtered through Whatman filter paper No. 1, the mycelial mat was washed in distilled water to remove the traces of medium and gently pressed between the folds of blotting paper to remove the excess amount of water. Inoculum was prepared by mixing 10 g mycelial mat in 100 ml of sterilized distilled water and blending it for 30 seconds in a waring blender. Thus each 10 ml of this suspension contained 1.0 g of the fungus.

## INOCULATION TECHNIQUES:

Feeder roots of balsam seedling, just before inoculations were exposed by carefully removing the top layer of soil and the required quantity of nematode suspension and/or fungus inoculum was poured uniformly all around the exposed roots using sterilized pipette. Exposed roots were immediately covered by leveling the soil properly. Untreated and uninoculated plants served as control. Each treatment was replicated three times and suitably randomized on a glass house bench. Watering was done as and when required.

## EXPERIMENTS:

### STUDIES ON THE PATHOGENICITY OF *MELOIDOGYNE JAVANICA* AND *MACROPHOMINA PHASEOLINA* ON BALSAM:

In order to determine the inoculum threshold of root-knot nematode i.e capable of causing significant damage, the seedlings of balsam were inoculated with 250, 500, 1000, 2000, 4000 and 8000 freshly hatched juveniles of *M. javanica.* Similarly, to determine the inoculum threshold level *of M. phaseolina*, the seedlings of balsam were inoculated with 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 g mycelia + sclerotia of the fungus / plant.

### EFFECT OF INDIVIDUAL, CONCOMITANT AND SEQUENTIAL INOCULATION OF *MELOIDOGYNE JAVANICA* AND *MACROPHOMINA PHASEOLINA* ON PLANT GROWTH AND DISEASE DEVELOPMENT:

This experiment was conducted to find out whether the interaction is concomitant or sequential. The experiment consisted of six treatments as given below:

1. Uninoculated (control)
2. Inoculated with *M. phaseolina*
3. Inoculated with *M. javanica*
4. *Macrophomina phaseolina* inoculated 15 days prior to *M. javanica*
5. *Meloidogyne javanica* inoculated 15 days prior to *M. phaseolina*
6. Concomitantly inoculated with *M. javanica* and *M. phaseolina*

## EFFECT OF INTERACTIONS OF DIFFERENT INOCULUM LEVELS OF *M. JAVANICA* AND *M. PHASEOLINA* ON BALSAM:

Besides inoculating test plants with either *M. javanica* or *M. phaseolina,* various combinations of concomitant inoculations of two pathogens using different inoculum levels of each were designed as given in the following table:

**Table - : INOCULATION SCHEDULE**

| Treatment No. | No. of juveniles/plant | Mycelia + Sclerotia in g /plant |
|---|---|---|
| 1. | 200 | - |
| 2. | 400 | - |
| 3. | 800 | - |
| 4. | - | 0.25 |
| 5. | - | 0.50 |
| 6. | - | 0.75 |
| 7. | 200 | 0.25 |
| 8. | 400 | 0.25 |
| 9. | 800 | 0.25 |
| 10. | 200 | 0.50 |
| 11. | 400 | 0.50 |
| 12. | 800 | 0.50 |
| 13. | 200 | 0.75 |
| 14. | 400 | 0.75 |
| 15. | 800 | 0.75 |

## LIFE-CYCLE OF *MELOIDOGYNE JAVANICA* ON BALSAM IN PRESENCE AND ABSENCE OF *MACROPHOMINA PHASEOLINA*:

Penetration and life cycle of *M. javanica* were studied on balsam. The present investigations were carried out in months of August-September and the prevailing

temperature during this period ranged between 35 to 37°C. Two week old seedlings of balsam were transplanted into 6" earthen pots containing 1 kg sterilized soil + river sand + farmyard manure (3:1:1) mixture. After one week of transplantation, one set of 100 seedlings were simultaneously inoculated with freshly hatched 1000 second stage juveniles of *M. javanica* and 1 g fungal suspension of *M. phaseolina* and the other set of 100 seedlings were individually inoculated with *M. javanica* served as control.

Observations on penetration and developmental stages of the nematode were recorded from three seedlings to each set after every 24 hrs (first being after 12 hrs) and continued till the completion of life-cycle. The complete root system of the seedling was carefully removed from the soil, washed gently in tap water and stained in 0.05% boiling lactophenol acid fuchsin (Franklin, 1949) followed by washing in tap water and keeping in plain lactophenol fur further differentiation.

Developmental stages of the nematode were identified (A, B, C, D and E) as described by Traintaphyllou and Hirschmann (1960):

A = pre parasitic II$^{nd}$ stage juvenile (filiform shaped)

B = parasitic II$^{nd}$ stage juvenile (spindle shaped)

C = III$^{rd}$ and IV$^{th}$ stage of juvenile (sausage shaped)

D = moulted IV$^{th}$ stage juvenile (moulted sausage shaped)

E = adults (females and males) sac and filiform shaped, respectively.

**EFFECT OF DIFFERENT DILUTIONS FUNGAL FILTRATE OF ROOT-ROT FUNGUS *MACROPHOMINA PHASEOLINA* ON HATCHING AND MORTALITY OF ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA IN VITRO*:**

Effect of culture filtrate of Root-rot fungus viz. *M. phaseolina*, isolated from the balsam root was studied on the hatching and mortality of root-knot nematode, *M. javanica*. *Macrophomina phaseolina* was grown for 15 days in 150 ml of Richard's medium. Culture filtrate was obtained by filtering 15 days old culture through Whatman filter paper No. 1. Filtrate thus obtained was clarified by centrifugation at 6000 rpm for 15 minutes and taken as standard solution 'S'. Different dilutions viz., S/2, S/10, S/100 and S/1000 of culture filtrate was prepared by adding required amount

of distilled water to standard solution 'S' Sterilized Petridishes of 5 cm diameter were separately pipetted with 5 ml of each dilution of fungal filtrate. Two drops of 0.1% solution of streptomycin sulphate were added to each dish for avoiding bacterial contamination. Five sterilized healthy eggmases of nearly uniform size of *M. javanica* were transferred to each dish. The eggmasses placed in sterilized distilled water served as control. All Petridishes were kept at 25 °C in an incubator. Total number of hatched out larvae in each Petridish was counted after 5 days under stereoscopic microscope.

For determining the effect of fungal filtrate on mortality of second stage juveniles of *M. javanica*, one hundred freshly hatched sterilized juveniles were transferred to 5 cm diameter Petridishes containing 5 ml filtrate of different dilutions (S, S/2, S/10, S/100 and S/1000) of fungus. Equal number of second stage juveniles were also transferred to separate Petridishes containing sterilized water to served as control. After 24, 48, 72, 96 hours the number of immobilized nematodes were counted under stereoscopic microscope. Apparently immobilized nematodes were first transferred to distilled water for an hour to ascertain their mortality. If they failed to regain mobility, they were considered dead.

**EFFECT OF ROOT-EXTRACTS OF BALSAM INFECTED WITH DIFFERENT INOCULUM LEVELS ON *MELOIDOGYNE JAVANICA* ON THE GROWTH OF *MACROPHOMINA PHASEOLINA IN VITRO*:**

To determine the effect of root-extract on the growth and sclerotial formation of *M. phaseolina*, 50g either healthy roots or galled roots were collected from balsam plants inoculated with different inoculum levels viz.250,500,1000,2000,4000 and 8000$J_2$ / plant.

These roots were separately macerated and blended in 50 ml distilled water and filtered through Whatman filter paper No. 1 to obtain the root extract. The root-extract was separately amended into the Richard's medium in the ratio of 1:1. The fungus grown in unamended Richard's medium served as control. These flasks were inoculated with *M. phaseolina* and incubated at 28±2°C. After 15 days mycelial mats were obtained, gently pressed between the folds of bloating paper to remove the excess amount of water and weighted. For determining the number of sclerotia /g mycelial mat, 1 ml fungal suspension poured into the counting dish and number of sclerotia were counted under the microscope.

## RECORDING OF OBSERVATIONS:

### PLANT GROWTH DETERMINATION:

Plants were uprooted after 60 days of inoculation and roots were washed thoroughly in slow running tap water. Utmost care was taken to avoid loss and injury of root system during the entire operation. For measuring length and dry weight, the plants were cut with a sharp knife just above the base of root emergence. Length of shoot and root was recorded in centimeters from the cut end to the tip of first leaf and the longest root respectively. For measuring dry weight, the shoot and root were kept in envelops separately for drying in an oven running at 80°C for 24 hours and the weight was recorded in grams. For interpretation of results, the reduction in plant growth was calculated in terms of percentage dry weight reduction.

### CHLOROPHYLL CONTENT:

The chlorophyll content in the fresh leaf was estimated as suggested by Mac Kinney (1941). One gram of finely cut fresh leaves of balsam was ground to a fine pulp using a mortar and pestle after pouring 20 $cm^3$ of 80% acetone. The mixture was centrifuged at 5000 rpm, for 5 minutes. The supernatant was collected in 100 $cm^3$ volumetric flask. The residue was washed three times, using 80% acetone. Each washing was collected in the same volumetric flask and volume was made upto the mark, using 80% acetone. The absorbance was read at 645 and 663 nm against the (80% acetone) blank on spectrophotometer. The chlorophyll content present in the extract (mg/g tissue) was calculated using the following equation:

$$\text{mg chlorophyll 'a' per g tissue} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll 'b' per g tissue} = 22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll} = 20.2\,(A_{645}) + 3.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

A = absorbance at specific wavelengths

V = final volume of chlorophyll extract in 80% acetone

W = fresh mass of tissue, used for extraction

**ROOT-KNOT AND ROOT-ROT ESTIMATION:**

The galls produced by root-knot nematode were estimated by counting the number of galls per root system. The percentage of rotting of root-system was also determined.

**NEMATODE POPULATION ESTIMATION:**

For extraction of nematodes, the soil from each treatment was mixed thoroughly and a sub-sample of 200 gm soil was processed through sieves according to Cobb's shifting and gravity method followed by Baermann funnel technique. Each suspension was collected in a beaker and volume made up to 100 ml. For proper distribution of nematodes, the suspension was bubbled with the help of pipette and 2 ml suspension from each sample was drawn and transferred to a counting dish. The number of nematodes were counted in three replicates for each sample. Mean of three such countings was calculated and the final population of nematodes/kg soil was determined.

To estimate the nematode population in roots, 1.0 g root from each replicate was macerated with enough water in an electrically operated waring blender for about 30 to 40 seconds. The macerate was collected in a beaker and volume made upto 100 ml. The nematode population was counted as described above. Reproduction factor (R) of nematode was calculated by the formula R = Pf / Pi, where "Pf" represented the final and "Pi" initial population of the nematode.

**STATISTICAL ANALYSIS:**

The data obtained were analyzed statistically and significance was calculated at P = 0.05 and P = 0.01 levels of probability.

# Results

# RESUSLTS

## STUDIES ON THE PATHOGENECITY OF ROOT-KNOT NEMATODE (*MELOIDOGYNE JAVANICA*) ON BALSAM:

It is evident form the data presented in Tables-1.1&1.2 that the reduction in plant growth characters of balsam was directly proportional to the inoculum levels of *M. javanica* i.e. with increasing the inoculum levels from 250 to 8000 second stage juveniles of *Meloidogyne javanica* ($J_2$) per plant, there was a corresponding increase in the reduction of plant growth characters of balsam. The inoculation of plants with 250, 500, 1000, 2000, 4000 and 8000 $J_2$ / plant resulted in 1.0, 2.9, 24.0, 28.8, 32.0 and 34.6% reduction in plant growth, respectively as compared to control. Similarly, in the corresponding treatments the reduction in the number of flowers was recorded as 4.3, 5.8, 25.5, 32.8, 35.7 and 40.8%, reduction in leaf area was 5.3, 8.4, 30.0, 34.6, 44.6 and 50.0, reduction in the number of pods / plant was 2.5, 5.9, 23.7, 34.7, 39.8 and 43.2, and reduction in total chlorophyll contents was 3.5, 6.3, 31.9, 33.6, 35.4 and 39.2% as compared to control. However, the inoculum levels upto 500 $J_2$/plant did not show significant reduction in plant growth characters as compared to control. Although, the significant reduction in plant growth was recorded at and above 1000 $J_2$/plant. Further, it was observed that the reduction in plant growth was not significant between the inoculum levels of 2000 and 4000 $J_2$, and 4000 and 8000 $J_2$ / plant.

A significant linear relationship was found between the initial population (Pi) and the final population (Pf) of *M. javanica*. The multiplication of root-knot nematode significantly reduced with the increase in the inoculum levels. The reproduction factor wass highest (15.8%) at the minimum inoculum level (250 $J_2$/plant) and lowest (2.1%) at the maximum inoculum level (800$J_2$/plant). Thus, the rate of nematode multiplication showed a declining trend with the increasing in the initial inoculum levels suggesting it to be a density dependent phenomenon. Similarly, there was a significant increase in the number of galls per root system with increase in the inoculum levels. The number of galls/root system was recorded as 21, 46, 105, 140, 159 and 172 at the inoculum levels of 250, 500, 1000, 2000, 4000 and

Table 1.1: Studies on the pathogenicity of *Meloidogyne javanica* on balsam.

| Inoculum levels | Plant length (cm) | | | Plant fresh weight (g) | | | Plant dry weight (g) | | | Percentage reduction over control | No. of galls/ root system |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | | |
| 0 | 98.6 | 29.6 | 128.2 | 216 | 69.0 | 285 | 69.2 | 18.2 | 87.4 | 0.0 | 0 |
| 250 | 97.2 | 28.8 | 126.0 | 212.4 | 67.8 | 280.2 | 69.3 | 17.2 | 86.5 | 1.0 | 21 |
| 500 | 96.2 | 28.1 | 124.3 | 211.7 | 67.5 | 279.2 | 67.9 | 16.9 | 84.8 | 2.97 | 46 |
| 1000 | 74.3 | 21.5 | 95.8 | 167.6 | 51.6 | 219.2 | 53.0 | 13.4 | 66.4 | 24.0 | 105 |
| 2000 | 72.3 | 21.3 | 93.6 | 160.6 | 48.7 | 209.3 | 49.8 | 12.4 | 62.2 | 28.8 | 140 |
| 4000 | 70.4 | 20.2 | 90.6 | 148 | 45.8 | 193.8 | 47.6 | 11.8 | 59.4 | 32.0 | 159 |
| 8000 | 69.2 | 19.2 | 88.4 | 146 | 44.0 | 190.0 | 45.8 | 11.3 | 57.1 | 34.6 | 172 |
| C.D.(P=0.05) | | | 3.92 | | | 6.10 | | | 3.37 | | 9.57 |
| C.D. (P=0.01) | | | 5.94 | | | 9.23 | | | 5.11 | | 15.00 |

Table - 1.2 : Studies on the pathogenicity of *Meloidogyne javanica* on balsam.

| Inoculum levels | Leaf area ($cm^2$) | Percentage reduction over control | No. of flowers/ plant | Percentage reduction over control | No. of Pods/ plant | Percentage reduction over control | Chl a (mg/g) | Percentage reduction over control | Chl b(mg/g) | Percentage reduction over control | Total Chl ( (mg/g) | Percentage reduction over control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 13 | 0.0 | 137 | 0.0 | 118 | 0.0 | 1.62 | 0.0 | 1.23 | 0.0 | 2.85 | 0.0 |
| 250 | 12.3 | 5.3 | 131 | 4.37 | 115 | 2.5 | 1.54 | 4.9 | 1.15 | 6.50 | 2.75 | 3.5 |
| 500 | 11.9 | 8.4 | 129 | 5.83 | 111 | 5.9 | 1.50 | 7.4 | 1.10 | 10.5 | 2.67 | 6.3 |
| 1000 | 9.1 | 30.0 | 102 | 25.5 | 90.0 | 23.7 | 1.07 | 33.9 | 0.79 | 35.7 | 1.94 | 31.9 |
| 2000 | 8.5 | 34.6 | 92 | 32.8 | 77.0 | 34.7 | 1.04 | 35.8 | 0.76 | 38.2 | 1.89 | 33.6 |
| 4000 | 7.2 | 44.6 | 88 | 35.7 | 71.0 | 39.8 | 1.0 | 38.2 | 0.71 | 42.2 | 1.84 | 35.4 |
| 8000 | 6.5 | 50.0 | 81 | 40.8 | 67.0 | 43.2 | 0.95 | 41.3 | 0.69 | 43.9 | 1.73 | 39.2 |
| C.D. (P= 0.05) | 1.76 | | 8.87 | | 8.35 | | 0.29 | | 0.25 | | 0.88 | |
| C.D. (P= 0.01) | 2.67 | | 11.92 | | 12.64 | | 1.95 | | 0.38 | | 1.30 | |

Table 1.3: Studies on the pathogenicity of *Meloidogyne javanica* on balsam.

| Inoculum levels | Nematode population / pot | | | Reproduction Factor (R=Pf/Pi) |
|---|---|---|---|---|
| | Juveniles | Females | Total | |
| 0 | 0 | 0 | 0 | 0 |
| 250 | 3815 | 135 | 3950 | 15.8 |
| 500 | 6207 | 293 | 6500 | 13.0 |
| 1000 | 9576 | 436 | 10012 | 10.0 |
| 2000 | 14513 | 487 | 15000 | 7.5 |
| 4000 | 18292 | 598 | 18890 | 4.7 |
| 8000 | 16546 | 519 | 17065 | 2.1 |
| C.D. (P=0.05) | | | | 2.30 |
| C.D. (P=0.01) | | | | 3.60 |

8000 $J_2$/plant, respectively. It can be concluded from these results that the damaging threshold levels of *M. javanica* on balsam was found as 1000 $J_2$ / plant (Table-1.3).

**STUDIES ON THE PATHOGENECITY OF ROOT-ROT FUNGUS (*MACROPHOMINA PHASEOLINA*) ON BALSAM:**

The data presented in Tables-2.1 & 2.2 clearly revealed that there was no significant variation in plant growth parameters upto 1.0g mycelial mat of *Macrophomina phaseolina* as compared to control. Moreover, the reduction in plant growth parameters was increased with the increase in the inoculum levels ranged from 2.0g to 4.0g mycelial mat/plant. The reduction in plant growth was found as 15.6, 18.3 and 21.0% in the plants inoculated with 2.0, 3.0 and 4.0g fungus/plant, respectively. The reduction in plant growth was not significantly different between inoculum levels of 2.0 and 3.0g, and 3.0 and 4.0g mycelial mat/plant. Similarly, in the corresponding treatments the reduction in the number of flowers was recorded as 17.5, 21.8 and 24.8%, in leaf area was 27.6, 33.8 and 39.2%, in the number of pods was 19.4, 22.8 and 24.5%, and reduction in total chlorophyll contents was 20.9, 25.4 and 27.4%.

Similarly, the percentage of root-rot/root system was also increased with increase an in inoculum levels except at the lowest (0.25g mycelial mat /plant) inoculum level. At the lowest inoculum, there was no root-rot. The percentage of root-rot was recorded as 4.0, 7.3, 20.0, 28.5 and 34.8% in the plants inoculated with 0.50, 1.00, 2.00, 3.00 and 4.00g mycelial mat/plant, respectively. It can be concluded from these results that the economic threshold level of *M. phaseolina* on balsam was recorded as 2.0g mycelial mat/plant.

**STUDIES ON THE EFFECT OF INDIVIDAUL, CONCOMITANT AND SEQUENTIAL INOCULATION OF ROOT-KNOT NEMATODE AND ROOT-ROT FUNGUS ON PLANT GROWTH AND DISEASE DEVELOPMENT OF BALSAM:**

It is evident from the Figs. 1-5 and data presented in Tables-3.1& 3.2 that the inoculation of balsam seedlings with *M. javanica* and *M. phaseolina* individually, concomitantly and sequentially caused the significant reduction in plant growth characters as compared to uninoculated plants (control). The reduction in plant

Table - 2.1: Studies on the pathogenicity of *Macrophomina phaseolina* on balsam.

| Inoculum levels | Plant length (cm) | | | Plant fresh weight (g) | | | Plant dry weight (g) | | | Percent reduction over control | Percentage of rotting / root system |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | | |
| 0 | 98.6 | 29.6 | 128.2 | 216 | 69 | 285 | 69.2 | 18.2 | 87.4 | 0 | 0.0 |
| 0.25 | 103.9 | 31.4 | 135.3 | 218.6 | 71.8 | 290.4 | 70.5 | 18.8 | 89.3 | +2.1 | 0.0 |
| 0.50 | 102.5 | 31.6 | 134.1 | 217.3 | 71.4 | 288.7 | 69.9 | 18.4 | 88.3 | +1.0 | 4.0 |
| 1.00 | 96.9 | 28.1 | 125.0 | 216.1 | 69.1 | 285.2 | 66.5 | 17.9 | 84.4 | 3.4 | 7.3 |
| 2.00 | 87.1 | 25.4 | 112.5 | 187.4 | 57.4 | 244.8 | 58.5 | 15.2 | 73.7 | 15.6 | 20.0 |
| 3.00 | 85.7 | 24.8 | 110.5 | 185.5 | 56.1 | 241.6 | 57.0 | 14.4 | 71.4 | 18.3 | 28.5 |
| 4.00 | 85.3 | 23.2 | 108.5 | 181.6 | 55.5 | 237.1 | 54.8 | 14.2 | 69.0 | 21.0 | 34.8 |
| C.D. (P = 0.05) | | | 11.0 | | | 5.67 | | | 5.20 | | |
| C.D. (P = 0.01) | | | 16.6 | | | 8.58 | | | 7.88 | | |

Table - 2.2: Studies on the pathogenicity of *Macrophomina phaseolina* on balsam.

| Inoculum levels | Leaf area ($cm^2$) | Percentage reduction over control | No. of flowers / plant | Percentage reduction over control | No. of pods / plant | Percentage reduction over control | Chl a (mg/g) | Percentage over control | Chl b (mg/g) | Percentage reduction over control | Total Chl (mg/g) | Percentage reduction over control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 13 | 0.0 | 137 | 0.0 | 118 | 0.0 | 1.32 | 0.0 | 1.12 | 0.0 | 2.44 | 0.0 |
| 0.25 | 12.3 | 5.3 | 135 | 1.4 | 115 | 2.5 | 1.27 | 3.7 | 1.05 | 6.2 | 2.26 | 7.3 |
| 0.50 | 12.5 | 3.8 | 133 | 2.9 | 116 | 1.6 | 1.28 | 3.0 | 1.08 | 3.5 | 2.30 | 5.7 |
| 1.00 | 11.8 | 9.2 | 131 | 4.3 | 114 | 3.3 | 1.23 | 6.8 | 1.01 | 9.8 | 2.23 | 8.6 |
| 2.00 | 9.4 | 27.6 | 113 | 17.5 | 95 | 19.4 | 1.05 | 20.4 | 0.81 | 27.6 | 1.93 | 20.9 |
| 3.00 | 8.6 | 33.8 | 107 | 21.8 | 91 | 22.8 | 0.99 | 25.0 | 0.77 | 31.2 | 1.82 | 25.4 |
| 4.00 | 7.9 | 39.2 | 103 | 24.8 | 89 | 24.5 | 0.96 | 27.2 | 0.74 | 33.9 | 1.77 | 27.4 |
| C.D.(P = 0.05) | 1.67 | | 7.03 | | 4.75 | | 0.17 | | 0.18 | | 0.38 | |
| C.D.(P = 0.01) | 2.54 | | 10.64 | | 6.91 | | 1.51 | | 0.27 | | 0.57 | |

Table - 3.1: Studies on the effect of individual, concomitant and sequential inoculation of *Meloidogyne javanica* and *Macrophomina phaseolina* on plant growth and disease development.

| Inoculum levels | Plant length (cm) | | | Plant fresh weight (g) | | | Plant dry weight (g) | | | Percentage reduction over control |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | |
| 0 | 98.6 | 29.6 | 128.2 | 216 | 69.0 | 285 | 69.2 | 18.2 | 87.4 | 0.0 |
| Fungus(F) | 89.0 | 25.9 | 114.9 | 201.1 | 62.5 | 263.6 | 61.7 | 15.7 | 77.4 | 11.4 |
| Nematode (N) | 76.2 | 21.8 | 98.0 | 171.3 | 52.7 | 224.0 | 51.3 | 12.9 | 64.2 | 26.5 |
| F → N | 68.2 | 19.2 | 87.4 | 156.2 | 47.8 | 204.0 | 45.8 | 11.4 | 57.2 | 34.5 |
| N + F | 57.9 | 16.4 | 74.3 | 129.6 | 39.1 | 168.7 | 39.1 | 9.6 | 48.7 | 44.2 |
| N→F | 54.4 | 15.3 | 69.7 | 127.5 | 36.9 | 164.4 | 37.6 | 9.2 | 46.8 | 46.4 |
| C.D. (P=0.05) | | | 4.71 | | | 6.93 | | | 4.04 | |
| C.D. (P=0.01) | | | 7.39 | | | 10.8 | | | 6.34 | |

Table - 3.2: Studies on the effect of individual, concomitant and sequential inoculation of *Meloidogyne javanica* and *Macrophomina phaseolina* on plant growth and disease development.

| Inoculum levels | Leaf area ($cm^2$) | Percentage reduction over control | No. of flowers/ plant | Percentage reduction over control | No. of Pods/ plant | Percentage reduction over control | Chl a (mg/g) | Percentage reduction over control | Chl b (mg/g) | Percentage reduction over control | Total Chl (mg/g) | Percentage reduction over control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 13 | 0.0 | 137 | 0.0 | 118 | 0.0 | 0.76 | 0.0 | 0.56 | 0.0 | 1.36 | 0.0 |
| Fungus (F) | 11.4 | 12.3 | 122 | 10.9 | 100 | 15.2 | 0.65 | 14.4 | 0.47 | 16.0 | 1.21 | 11.0 |
| Nematode(N) | 10 | 23.0 | 106 | 22.6 | 73 | 38.1 | 0.53 | 30.2 | 0.38 | 32.1 | 1.01 | 25.7 |
| F →N | 8.3 | 36.1 | 83 | 39.4 | 63 | 46.6 | 0.46 | 39.4 | 0.33 | 41.0 | 0.91 | 33.0 |
| N + F | 6.5 | 50.0 | 66 | 51.8 | 52 | 55.9 | 0.38 | 50.0 | 0.26 | 53.5 | 0.79 | 41.9 |
| N→F | 5.7 | 56.1 | 53 | 61.3 | 46 | 61.0 | 0.36 | 52.6 | 0.25 | 55.3 | 0.74 | 45.5 |
| C.D.(P=0.05) | 1.54 | | 15.21 | | 9.23 | | 0.20 | | 0.10 | | 0.18 | |
| C.D.(P=0.01) | 2.41 | | 23.85 | | 14.47 | | 0.03 | | 0.30 | | 0.32 | |

Table- 3.3: Studies on the effect of individual, concomitant and sequential inoculation of *Meloidogyne javanica* and *Macrophomina phaseolina* on plant growth and disease development.

| Inoculum levels | Nematode population | | | | No. of galls | Percentage of rotting per root system |
|---|---|---|---|---|---|---|
| | Juveniles | Females | Total | Reproduction factor | | |
| O | 0 | 0 | 0 | 0 | 0 | 0 |
| Fungus (F) | 0 | 0 | 0 | 0 | 0 | 18.6 |
| Nematode (N) | 11710 | 407 | 12117 | 12.11 | 91 | 0 |
| F →N | 5021 | 382 | 5403 | 5.40 | 42 | 45.2 |
| N+F | 6359 | 345 | 6704 | 6.70 | 60 | 61.9 |
| N →F | 8699 | 207 | 8906 | 8.90 | 78 | 67.3 |
| C.D.(P=0.05) | | | | 2.35 | 6.38 | |
| C.D.(P=0.01) | | | | 4.31 | 9.02 | |

growth, leaf area, number of flowers, number of pods and total chlorophyll contents was recorded as 26.5, 23.0, 22.6, 38.1 and 25.7%, respectively in the plants individually inoculated with *M. javanica.* Similarly, the reduction in the corresponding parameters was found as 11.4, 12.3, 15.2, 10.9 and 11.0% as compared to control, in the plants inoculated with *M. phaseolina.*

Moreover, the greatest reduction in plant growth parameters was caused by the sequential inoculation of nematode 15 days prior to fungus followed by the simultaneous inoculation of *M. javanica* and *M. phaseolina*, and fungus inoculation 15 days prior to nematode. In the corresponding treatments the reduction in plant growth was recorded as 46.4, 44.2 and 34.5%, in leaf area was 56.1, 50.0 and 36.1%; in number of flowers was 61.3, 51.8, and 39.4%; in number of pods was 61.0, 55.9 and 46.6% and in chlorophyll contents was 45.5, 41.9 and 33.0%, as against control.

The intensity of root-rot/root system caused by *M. phaseolina* was increased in the presence of root-knot nematode *M. javanica* as compared to when *M. phaseolina* inoculated individually. The highest root-rot (67.3%) was recorded in the plants sequentially inoculated with *M. javanica* 15 days prior to fungus followed by in the plants inoculated with *M. javanica* and *M. phaseolina* simultaneously (61.9%) and *M. phaseolina* 15 days prior to *M. javanica* (45.2).

The root-knot nematode multiplication and the development of galls/root system were significantly reduced in presence of *M. phaseolina.* The greatest reproduction factor (12.11) and number of galls (91.0) per root system were recorded in the plants inoculated with *M. javanica* alone. However, on the other hand, the reproduction factor was recorded as 5.40, 6.70 and 8.90 and number of galls/root system was found as 42, 60 and 78 in the plants inoculated with fungus 15 days prior to nematode, nematode + fungus, and nematode 15 days prior to fungus, respectively (Table 3.3).

Basically, *M. javanica* was the parasite of roots. The above ground symptoms, therefore, due to slow debility of roots in its function of nutrients and water uptake and translocation. Infected plants were dwarfed with few smaller and yellowish leaves (Fig. 1). The below ground symptoms on the roots with small galls which in case of multiple infection on the nearby tissues, small galls may coalesce to form large gall in some cases. Sometimes, branched, small and fine roots also present on

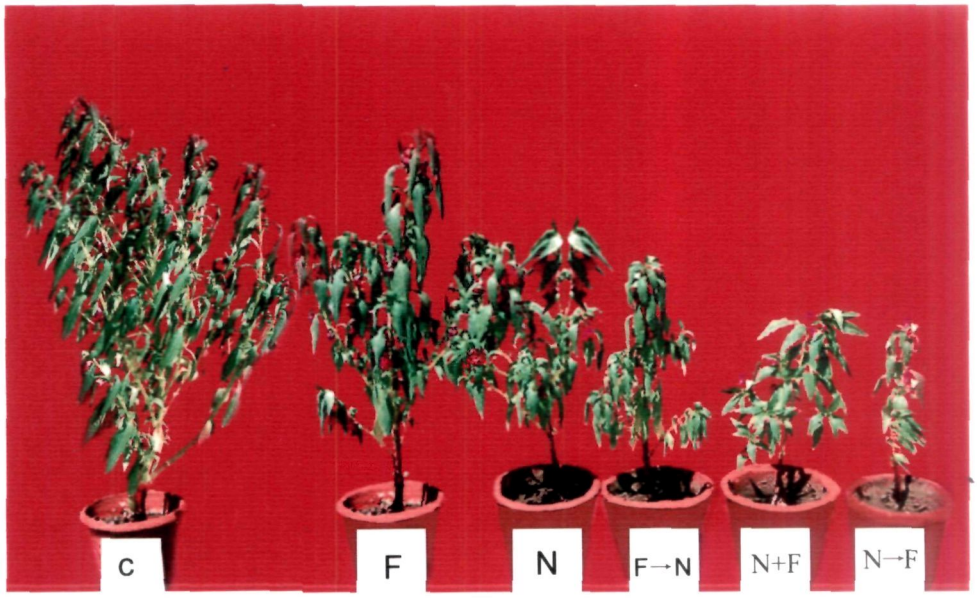


**Fig-1:**Effect of individual, concomitant and sequential inoculation of *Meloidogyne javanica* and *Macrophomina phoseolina* on the growth of balsam.

**C** = Control

**F** = *M. phaseolina*

**N** = *M. javanica*

**F → N** = *M. phaseolina 15 days* prior to *M. javanica*

**N + F** = *M. phaseolina* and *M. javanica*

**N→ F** = *M. javanica* 15 days prior to *M. phaseolina*


**Fig-2:** Effect of individual, concomitant and sequential inoculation of *Meloidogyne javanica* and *Macrophomina phoseolina* on the growth of root and disease development.

**C** = Control
**F** = *M. phaseolina*
**N** = *M. javanica*
**F → N** = *M. phaseolina 15 days* prior to *M. javanica*
**N + F** = *M. phaseolina* and *M. javanica*
**N → F** = *M. javanica* 15 days prior to *M. phaseolina*

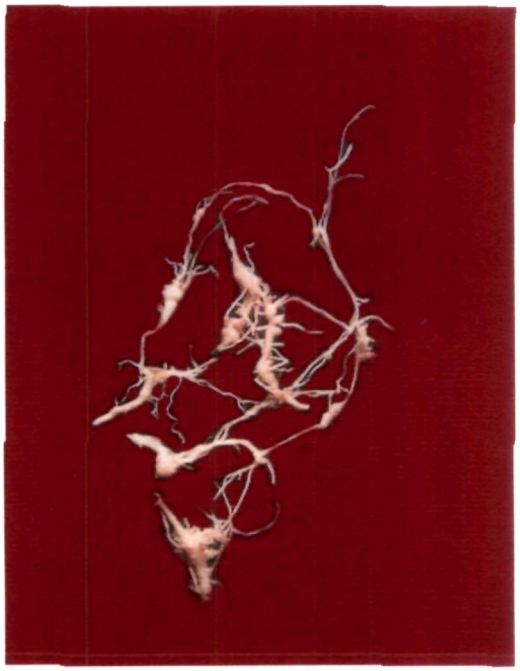

**Fig-3:** Showing the galls produced by *Meloidogyne javanica* on the roots of balsam.

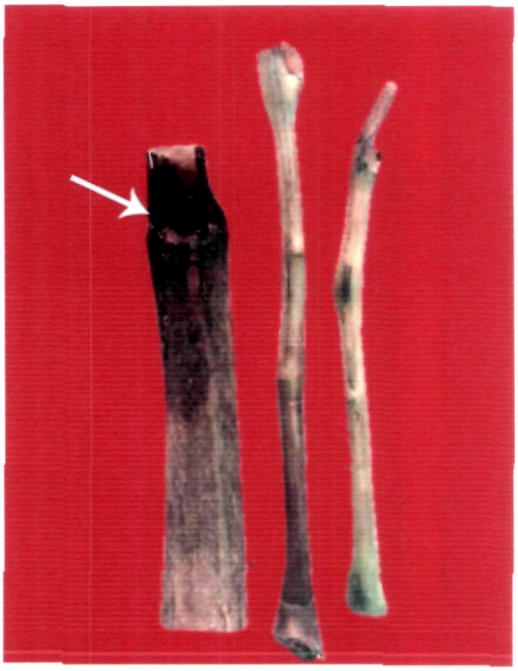

**Fig-4:** Showing the stem-rotting caused by *Meloidogyne javanica* and *Macrophomina phaseolina* on balsam.

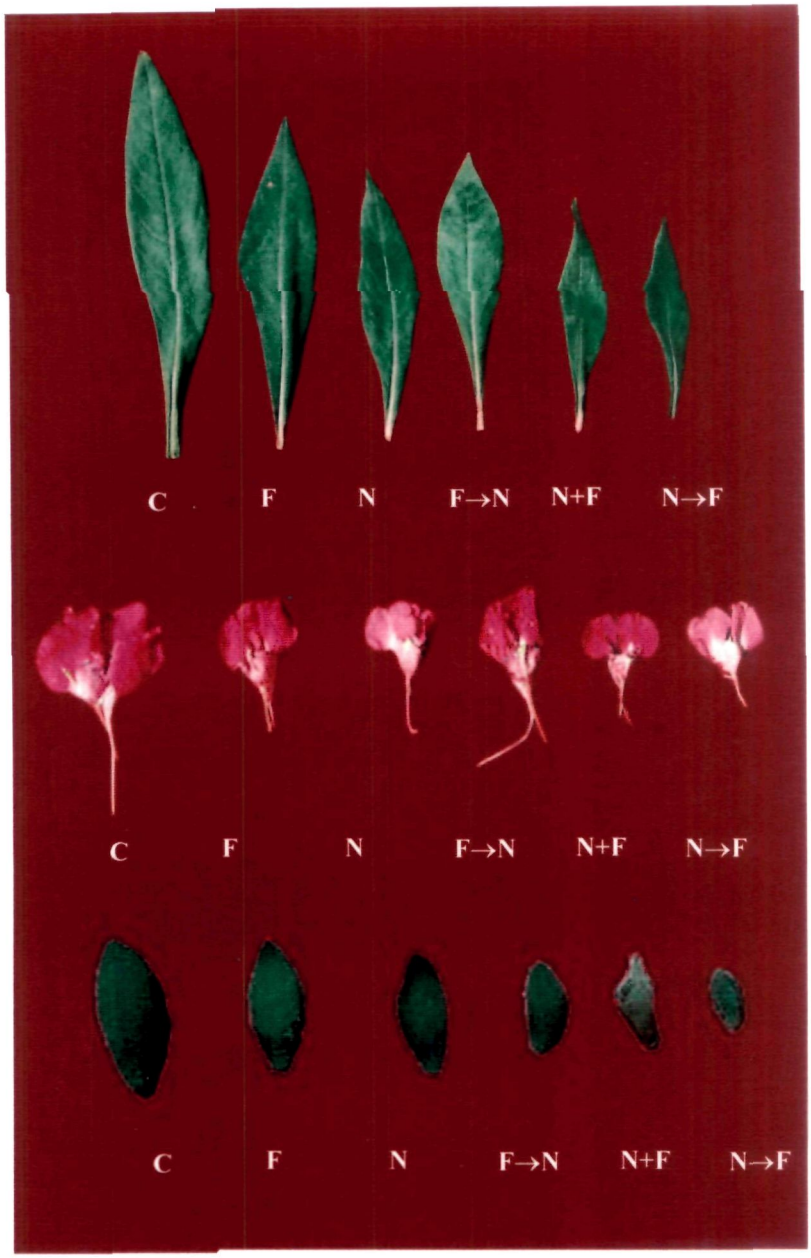


**Fig-5:** Effect of individual, concomitant and sequential inoculation of *Meloidogyne javanica* and *Macrophomina phoseolina* on the development of leaves, flowers and pods.

**C** = Control

**F** = *M. phaseolina*

**N** = *M. javanica*

**F → N** = *M. phaseolina 15 days* prior to *M. javanica*

**N + F** = *M. phaseolina* and *M. javanica*

**N→ F** = *M. javanica* 15 days prior to *M. phaseolina*

the surface of galls (Fig. 3). Overall, the root galls produced by nematodes were not very prominent. The growth of tap root was suppressed i.e. blinding of the main tap root and lateral growth was profused (Fig. 2). At the highest inoculum level (8000 $J_2$ / plant) the diseased plant showed temporary day time wilting during hot hours even in the presence of enough soil moisture.

**EFFECT OF INTERACTIONS OF DIFFERENT INOCULUM LEVELS OF ROOT-KNOT NEMATODE AND ROOT-ROT FUNGUS ON PLANT GROWTH AND DISEASE DEVELOPMENT.**

It is evident from the data presented in Tables-4.1 & 4.2 that with an increase in the inoculum levels of either *M. javanica* (from 200 to 800 $J_2$/plant) or *M. phaseolina* (from 0.25 to 0.75g mycelial mat/plant) there was no significant variation in plant growth parameters as compared to control except at the inoculum level of 800 $J_2$ of *M. javanica* plant. A significant linear relationship was observed between the initial and final nematode population. The reproduction factor of *M. javanica* was highest (14.7) when the initial inoculum was lowest (200 $J_2$/plant) but, lowest (9.47) when initial inoculum was highest (800 $J_2$/plant).

The simultaneous inoculation of plants with different inoculum levels of nematode and fungus synergistically increased root-rot and reduction in plant growth characters with the increase in the inoculum levels of *M. javanica* and *M. phaseolina* as compared to their individual inoculation. It was interesting to note that, significant reduction in plant growth (12.5) was observed even when the seedlings were concomitantly inoculated with lowest inocula (200 juvenile of *M. javanica* + 0.25g fungus of *M. phaseolina).* The greatest reduction in plant growth (43.7) and the maximum root-rot (63%) was observed when the plants received the highest inocula of nematode and fungus (800$J_2$ + 0.75g mycelial mat/plant). The reduction in plant growth was recorded as 39.3, 29.1, 26.6, 25.4, 20.7, 18.3, 16.2 and 12.5% and the intensity of root-rot was recorded as 59, 48, 42, 37, 34, 26, 20 and 14% in the plants inoculated with 400 $J_2$ + 0.75 fungus, 800 $J_2$ + 0.50 fungus, 200 $J_2$ + 0.75 fungus, 400 $J_2$ + 0.5g fungus, 800 $J_2$ + 0.25g fungus, 400 $J_2$ + 0.25g fungus, 200 $J_2$ + 0.5g and 200 $J_2$ + 0.25g fungus, respectively.

The rate of root-knot nematode multiplication and number of galls/root system were significantly reduced in the presence of highest inoculum level of the fungus i.e.

Table- 4.1: Studies on the effect of interactions of different inoculum levels of *Meloidogyne javanica* and *Macrophomina phaseolina* on plant growth.

| Inoculum levels | Plant length (cm) | | | Plant fresh weight(g) | | | Plant dry weight(g) | | Total | Percentage reduction over control |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | | |
| 0 | 98.6 | 29.6 | 128.2 | 216 | 69.0 | 285 | 69.2 | 18.2 | 87.4 | 0.0 |
| 200 | 97.5 | 28.9 | 126.4 | 212 | 66.1 | 278.1 | 68.8 | 17.4 | 86.2 | 1.3 |
| 400 | 96.4 | 27.3 | 123.7 | 209.1 | 65.3 | 274.4 | 68.1 | 17.2 | 85.3 | 2.4 |
| 800 | 88.4 | 26.2 | 114.6 | 188.8 | 58.5 | 247.3 | 57.7 | 15.0 | 72.7 | 16.8 |
| 0.25 | 97.7 | 28.8 | 126.5 | 212.8 | 67.9 | 280.7 | 67.9 | 17.6 | 85.5 | 2.1 |
| 0.50 | 97.6 | 28.6 | 126.2 | 211.6 | 66.8 | 278.4 | 67.6 | 17.5 | 85.1 | 2.6 |
| 0.75 | 97.3 | 28.4 | 125.7 | 210.3 | 66.4 | 276.7 | 67.4 | 17.4 | 84.8 | 2.9 |
| 200+0.25 | 85.0 | 28.8 | 113.8 | 199.4 | 61.9 | 261.3 | 61.2 | 15.2 | 76.4 | 12.5 |
| 400+0.25 | 78.9 | 22.8 | 101.7 | 182.1 | 56.4 | 238.5 | 56.9 | 14.5 | 71.4 | 18.3 |
| 800+0.25 | 78.0 | 22.5 | 100.5 | 177.2 | 54.2 | 231.4 | 55.4 | 13.9 | 69.3 | 20.7 |
| 200+0.5 | 77.7 | 22.2 | 99.9 | 173.3 | 52.9 | 226.2 | 59.7 | 13.5 | 73.2 | 16.2 |
| 400+0.5 | 76.8 | 21.9 | 98.7 | 173.1 | 52.0 | 225.1 | 51.9 | 13.3 | 65.2 | 25.4 |
| 800+0.5 | 73.8 | 21.4 | 95.2 | 163.9 | 49.5 | 213.4 | 49.1 | 12.8 | 61.9 | 29.1 |
| 200+0 75 | 71.5 | 20.8 | 92.3 | 159.8 | 48.7 | 208.5 | 51.7 | 12.4 | 64.1 | 26.6 |
| 400+0.75 | 69.1 | 19.9 | 89.0 | 158.2 | 47.5 | 205.7 | 33.6 | 19.4 | 53.0 | 39.3 |
| 800+0.75 | 64.5 | 18.5 | 83.0 | 147.2 | 43.7 | 190.9 | 40.6 | 8.6 | 49.2 | 43.7 |
| C.D.(P=0.05) | | | 2.66 | | | 3.69 | | | 2.32 | |
| C.D.(P=0.01) | | | 3.71 | | | 5.14 | | | 3.23 | |

Table- 4.2: Studies on the effect of interactions of different inoculum levels of *Meloidogyne javanica* and *Macrophomina phaseolina* on nematode multiplication and disease development.

| Inoculum levels | Nematode population/pot | | | $R = \frac{Pf}{Pi}$ | No. of galls/ root system | Percentage of rotting / root system |
|---|---|---|---|---|---|---|
| | Juveniles | Females | Total | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 200 | 2854 | 87 | 2941 | 14.7 | 17 | 0 |
| 400 | 4747 | 174 | 4921 | 12.3 | 27 | 0 |
| 800 | 7280 | 297 | 7577 | 9.4 | 39 | 0 |
| 0.25 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.50 | 0 | 0 | 0 | 0 | 0 | 5.6 |
| 0.75 | 0 | 0 | 0 | 0 | 0 | 8.3 |
| 200+0.25 | 2651 | 123 | 2774 | 13.8 | 19 | 14 |
| 400+0.25 | 4154 | 177 | 4331 | 10.8 | 26 | 20 |
| 800+0.25 | 6328 | 393 | 6721 | 8.4 | 37 | 26 |
| 200+0.5 | 2448 | 115 | 2563 | 12.8 | 16 | 34 |
| 400+0.5 | 3791 | 196 | 3987 | 9.9 | 24 | 37 |
| 800+0.5 | 5345 | 376 | 5721 | 7.1 | 35 | 42 |
| 200+0.75 | 1590 | 101 | 1691 | 8.4 | 11 | 48 |
| 400+0.75 | 2545 | 158 | 2703 | 6.7 | 19 | 59 |
| 800+0.75 | 3430 | 313 | 3743 | 4.6 | 27 | 63 |
| C.D.(P=0.05) | | | | 2.51 | 4.16 | |
| C.D.(P=0.01) | | | | 2.97 | 6.64 | |

0.75 g mycelial mat/plant as compared to their individual inoculation of *M. javanica*. Moreover, there were no significant differences in the reproduction factor and number of galls in presence of lower inocula viz., 0.25g and 0.50g mycelial mat/plant. The greatest reduction in reproduction factor (Rf = 4.67) was observed in the presence of highest inoculum level of the fungus. The reproduction factor was found as 13.8, 10.8, 8.4, 12.8, 9.9, 7.1, 8.4, 6.7 and 4.6 in the plants inoculated with 200 $J_2$ + 0.25 g , 400 $J_2$ + 0.25 g, 800 $J_2$ + 0.25 g, 200 $J_2$ + 0.5 g, 400 $J_2$ + 0.5 g, 800 $J_2$ + 0.5 g, 200 $J_2$ + 0.75 g, 400 $J_2$ + 0.75 g and 800 $J_2$ of *M. javanica* + 0.75 g mycelial mat of *M. phaseolina*, respectively. In the corresponding treatments the number of galls/root system was also recorded as 19, 26, 37, 16, 24, 35, 11, 19 and 27.

## STUDIES ON THE LIFE CYCLE OF ROOT-KNOT NEMATODE (*MELOIDOGYNE JAVANICA*) ON BALSAM IN PRESENCE AND ABSENCE OF ROOT-ROT FUNGUS (*MACROPHOMINA PHASEOLINA*):

The data presented in Table-5 clearly showed that the penetration of second stage juveniles of *M. javanica* started within 12 hrs after inoculation and the rate of penetration gradually increased with the passage of time upto the 5$^{th}$ day in the plants inoculated with root-knot nematode alone. Mostly, the penetration of second stage juveniles of *M. javanica* took place in the meristematic region but in some cases the juvenile also penetrated into the root tips and oriented themselves near the stelar region almost parallel to the longitudinal axis of the roots.

It was observed that on the 5$^{th}$ day of inoculation 80.4% nematodes were found in "A" stage, whereas, on 7$^{th}$ day only 5.0% nematodes were in "A" stage and 70.4% were recorded as "B" stage. Moreover, on the 12$^{th}$ day of inoculation most of the nematodes were traced in "C" stage (74.6%), only a few in "B" stage (6.2%), whereas, the nematodes of "A" stage was absent. On 25$^{th}$ day of inoculation further no nematodes of "A" and "B" stages were traced, but only few nematodes could be traced as "C" stage (7.0%) and majority of the nematodes were in "D" stage of development. Most of the developmental stages viz., "A", "B", "C" and "D" were absent on 19$^{th}$ day of inoculation except the "E" developmental stage (Adult females) of *M. javanica* (60.4%). Adult males (3.7%) were also found on 19$^{th}$ day after the inoculation.

Table - 5: Studies on the life cycle of *Meloidogyne javanica* on balsam in presence and absence of *Macrophomina phaseolina.*

| **Days after inoculation** | | **Developmental stages of root-knot nematode (%) / Treatments** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **A** | | **B** | | **C** | | **D** | | **E** | | **Male** | |
| Mj | Mj+Mp | Mj | Mj+Mp | Mj | Mj+Mp | Mj | Mj+Mp | Mj | Mj+Mp | Mj | Mj+Mp | Mj | Mj+Mp |
| 5 | 6 | 80.4 | 62.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | 10 | 5.0 | 9.3 | 70.4 | 58.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 12 | 18 | 0.0 | 4.7 | 6.2 | 8.4 | 74.6 | 55.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 15 | 23 | 0.0 | 0.0 | 0.0 | 5.2 | 7.0 | 10.8 | 63.2 | 40.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| 19 | 27 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.9 | 0.0 | 8.3 | 60.4 | 37.4 | 3.7 | 12.5 |

Average number of eggs/ eggmass = 387 (Mj) and 185 (Mj + Mp)

*Females formed (Percent) = 75.1 (Mj) and 46.5 (Mj+Mp)

*Males formed (Percent) = 4.60 (Mj) and 15.5 (Mj+Mp)

Deposition of gelatinous matrix was recorded 22$^{nd}$ day (Mj) and 30$^{th}$ day (Mj+Mp)

Deposition of eggs in eggmass was recorded on 23$^{rd}$ day (Mj) and 32$^{nd}$ day (Mj+Mp)

Emergence of second generation larvae was recorded on 25$^{th}$ day (Mj) and 33$^{rd}$ day (Mj+Mp) and the number of larvae / kg soil was 3048 and 1897, respectively.

Mj = *Meloidogyne javanica*

Mp = *Macrophomina phaseolina*

* *Values calculated from the number of larvae penetrated.*

Similarly, the penetration of juveniles in the roots of balsam plants started within 12 hrs after the simultaneous inoculation of *M. javanica* and *M. phaseolina*. The penetration of nematodes gradually increased with the passage of time till 6$^{th}$ day of inoculation. The mode of penetration of juveniles was similar as it was observed in the plants inoculated with *M. javanica* alone. The highest percentage (62.3%) of "A" stage was observed on 6$^{th}$ day of inoculation and no other stages of nematode were seen. However, most of the nematodes were found to be undergoing in "B" stage (58.8%) and very few in "A" stage (9.3%) on 10$^{th}$ day after inoculation. The highest percentage of "C" developmental stage (55.3%) of root-knot nematode was recorded on 18$^{th}$ day after inoculation, followed by 8.4 and 4.7% of in "B" and "A" stages, respectively. However, on 23$^{rd}$ day, no nematode was observed in "A" stage, while, very few in "B" and "C" stages (5.2 and 7.0%, respectively) and 40.7% nematodes in "D" stage of development and maturity were recorded. Adult males (12.5%) and females (37.4%) could be differentiated on 27$^{th}$ day of inoculation, however, some nematodes were also found in "C" stage 3.9% and in "D" stage (8.3) and no nematode was observed in "A" stage.

In *M. javanica* inoculated plants, 80.4% juveniles were penetrated into the roots of balsam as compared to 62.3% in *M. javanica* + *M. phaseolina* inoculated plants. Further, of the total juveniles that entered the roots, 75.1% developed into females in *M. javanica* inoculated plants as against 46.5% in *M. javanica* + *M. phaseolina* inoculated plants. Fecundity of the females was also found to be reduced with an average of only 185 eggs/eggmass in *M. javanica* + *M. phaseolina* inoculated plant as compared to 387 eggs per eggmass in *M. javanica* inoculated plants. The percentage of male formation of *M. javanica* was greater (12.5%) in presence of *M. phaseolina* as compared to when *M. javanica* was present alone (3.7%). The plants inoculated with *M. javanica* alone, showed the deposition of gelatinous matrix on 22$^{nd}$ day, eggs laying in eggmass was recorded on 23$^{rd}$ day and emergence of second stage juveniles was recorded on 25$^{th}$ day after inoculation. However, on the other hand, the corresponding stages of *M. javanica* in presence of *M. phaseolina* were recorded on 30$^{th}$ day, 32$^{nd}$ day and 33$^{rd}$ day. These results showed that the life cycle of *M. javanica* on balsam was completed within 25 days, whereas, the duration of life cycle was adversely affected in the presence of fungus (*M. phaseolina*) and it takes about

33 days to complete the life-cycle i.e. presence of *M. phaseolina* delayed the life-cycle of root-knot nematode (*M. javanica*) by 8 days.

**EFFECT OF DIFFERENT DILUTIONS OF FUNGAL FILTRATE OF *MACROPHOMINA PHASEOLINA* ON THE MORTALITY OF *MELOIDOGYNE JAVANICA IN VITRO*:**

The data presented in Table-6 clearly indicated that different dilutions of culture filtrate of *M. phaseolina* showed varied nematicidal effect to *M. javanica*. Percentage mortality of nematodes was directly proportional to the concentrations of filtrates and the period for which the nematodes were exposed. The rate of mortality was low in beginning but an appreciable increase was recorded after 24 hours of exposure to S, S/2 and S/10 dilutions. However, on the other hand, no mortality of root- knot nematode was recorded in S/100 and S/1000 concentrations even when juveniles exposed upto 96 hrs. The highest mortality of root-knot nematode was recorded in 'S' concentration after 96 hrs. of exposure. Moreover, the lowest percentage of mortality was observed in S/10 dilution at 24 hrs exposure period. The mortality of root-knot nematode in "S" concentration was recorded as 48, 54, 72 and 87% when juveniles were exposed to 24, 48, 72 and 96 hours, respectively. Similarly, in the corresponding exposure periods the mortality was recorded 30, 49, 58 and 62% in S/2 concentration, whereas, in S/10 concentration the mortality was 7, 13, 15, 22%. More than 50% mortality was recorded only in "S" and "S/2" when juveniles were exposed to 48 and 72 hours, respectively.

**EFFECT OF DIFFERENT DILUTIONS OF FUNGAL FILTRATE OF *MACROPHOMINA PHASEOLINA* ON THE HATCHING OF *MELOIDOGYNE JAVANICA* :**

The data presented in Table-7 clearly indicated that different concentration of culture filtrate of *M. phaseolina* showed significant inhibition in the juveniles emergence of *M. javanica* to varying degree except in the S/100 and S/1000 concentrations. There was a relative decrease in the hatching of *M. javanica* with the corresponding increase in the concentration of culture filtrate and the exposure period. The rate of hatching inhibition in the S, S/2 and S/10 concentration was low in the beginning but appreciably increase with the increase in the exposure period. The highest significant inhibition in the hatching of root-knot nematode was recorded in

Table-6: Studies on the effect of different dilution of fungal filtrate of *Macrophomina phaseolina* on the mortality of *Meloidogyne javanica*

| **Exposure Time (hrs)** | **Distilled water** | **Concentrations of fungal filtrate/Mortality (%)** | | | | | **C.D.** | |
|---|---|---|---|---|---|---|---|---|
| | | **S** | **S/2** | **S/10** | **S/100** | **S/1000** | **P=0.05** | **P=0.01** |
| 24 | 0 | 48 | 30 | 7 | 0 | 0 | 5.53 | 8.68 |
| 48 | 0 | 54 | 49 | 13 | 0 | 0 | 5.92 | 9.28 |
| 72 | 0 | 72 | 58 | 15 | 0 | 0 | 6.54 | 10.43 |
| 96 | 0 | 87 | 62 | 22 | 0 | 0 | 6.84 | 10.73 |
| C.D. (P=0.05) | | 5.51 | 6.10 | 3.39 | | | | |
| C.D. (P=0.01) | | 10.12 | 11.20 | 6.23 | | | | |

Table-7: Studies on the effect of different dilution of fungal filtrate of *Macrophomina phaseolina* on the hatching of *Meloidogyne javanica*

| *Exposure time* (hrs) | Distilled water | Concentrations of fungal filtrate/Egg hatch (%) | | | | | C.D. | |
|---|---|---|---|---|---|---|---|---|
| | | S | S/2 | S/10 | S/100 | S/1000 | P= 0.05 | P=0.01 |
| 24 | 55 | 33 | 40 | 50 | 62 | 55 | 4.38 | 7.26 |
| 48 | 106 | 48 | 64 | 98 | 121 | 103 | 4.91 | 9.12 |
| 72 | 188 | 73 | 111 | 168 | 123 | 193 | 6.05 | 10.32 |
| 46 | 238 | 90 | 134 | 241 | 295 | 293 | 2.25 | 3.95 |
| C.D. P = 0.05 | | 5.16 | 6.12 | 3.93 | 3.18 | 2.62 | | |
| C.D. P= 0.01 | | 9.31 | 9.24 | 5.97 | 6.21 | 3.99 | | |

'S' concentration at 96 hours, whereas, the lowest inhibition was found in S/10 concentration at 24 hours. The percentage reduction in the inhibition of hatching in 'S' concentration was recorded as 40.0, 54.7, 61.1 and 68.7 at 24, 48, 72 and 96 hours, respectively. Similarly, in the corresponding exposure time, the reduction in the inhibition of hatching was found as 27.2, 39.6, 50.9 and 53.4 in S/2 concentration, while, it was recorded as 9.0, 7.5, 10.6 and 16.3 in S/10 as compared to control.

However, on the other hand, it was interesting to note that S/100 concentration of culture filtrate showed the significant stimulatory effect on the juveniles hatch. Stimulation of hatching in the same dilution was observed as 12.7, 14.1, 18.6 and 23.9% at 24, 48, 72 and 96 hours, in comparison to control. Moreover, there was no significant variation in hatching of root-knot nematode was observed in S/1000 concentration as compared to control.

**EFFECT OF ROOT EXTRACT OF BALSAM INFECTED WITH DIFFERENT INOCULA OF *MELOIDOGYNE JAVANICA* ON THE GROWTH OF *MACROPHOMINA PHASEOLINA IN VITRO*:**

It is evident from the data presented in Table-8 that the incorporation of galled root-extract of balsam infected with *M. javanica* into Richards medium significantly stimulated the growth and sclerotia formation of *M. phaseolina* except the root-extract obtained from healthy roots and from plants infected with lowest inoculum (250 $J_2$/plant). The stimulatory effect of root-extract on the growth and sclerotial formation was directly correlated with the density of root-knot nematode infected the plants. The highest stimulatory effect was observed at the maximum inoculum level of root-knot nematodes where as the lowest was recorded in the Richard's medium amended with the root-extract of plant inoculated with 500 $J_2$/plant as compared to control.

The stimulatory effect of galled root extract on the mycelial growth was recorded as 3.4, 4.5, 23.5, 35.2, 59.9, 68.7 and 77.8% and in sclerotial formation was 2.2, 11.3, 27.2, 34.0, 38.6 and 43.1% in the Richard's medium added with galls root extracts collected from the plants inoculated with 250, 500, 1000, 2000, 4000 and 8000 $J_2$/plant, respectively as compared to control.

Table-8: Studies on the effect of root extract of balsam infected with different inocula of *Meloidogyne javanica* on the growth of *Macrophomina phaseolina in vitro*

| Treatments* | Mycelial weight (g) | % reduction (-) or stimulation (+) over control | No. of sclerotia /g mycelialmat | % reduction (-) or stimulation (+) over control |
|---|---|---|---|---|
| *RM | 35.2 | - | 44 | - |
| RM + $RE_1$ | 34.3 | -3.4 | 43 | -2.2 |
| RM + $RE_2$ | 36.8 | +4.5 | 45 | +2.2 |
| RM + $RE_3$ | 43.5 | + 23.5 | 49 | + 11.3 |
| RM+$RE_4$ | 47.6 | +35.2 | 56 | +27.2 |
| RM+$RE_5$ | 56.3 | +59.9 | 59 | +34.0 |
| RM+$RE_6$ | 59.9 | +68.7 | 61 | +38.6 |
| RM + $RE_7$ | 62.6 | +77.8 | 63 | +43.1 |
| C.D.(P= 0.05) | 2.79 | | 3.88 | |
| C.D.(P=0.01) | 4.13 | | 4.79 | |

*RM = Richards medium control
$RE_1$ = Root extracts from uninoculated plants
$RE_2$ = Root extracts from plant inoculated with 250 $J_2$
$RE_3$ = Root extracts from plant inoculated with 500 $J_2$
$RE_4$ = Root extracts from plant inoculated with 1000 $J_2$
$RE_5$ = Root extracts from plant inoculated with 2000 $J_2$
$RE_6$ = Root extracts from plant inoculated with 4000 $J_2$
$RE_7$ = Root extracts from plant inoculated with 8000 $J_2$

# *Discussion*

# DISCUSSION

Among the factors contributing towards damage to hosts by plant parasitic nematodes, the nematode species, their population levels and the kind of host are the major determinants. However, it would be practically unrealistic to evaluate the relationship of every nematode species associated with every cultivar under all possible environmental conditions, but some general assumptions can be indicated with the assistance of damage predicting models. One of the limiting factors in developing models that predict crop losses caused by nematodes and which generate advisory programmes, has been non-availability of data. Seinhorst's (1965) damage prediction model is one such model which has biological basis and is widely used for describing the relationship between nematode density and plant yield. Tolerance levels have been computed for several crops using this model and their relevance and importance in management decisions have been pointed out by Ferris (1980). Data on root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp.) have been largely subjected for studying the relationships and computing the tolerance levels (Seinhorst, 1965; Oostenbrink, 1966 and Vrain, 1982).

To determine the inoculum threshold level of root-knot nematode (*M. javanica*) and root-rot fungus (*M. phaseolina*) the seedlings of balsam were separately inoculated with different inoculum levels of *M. javanica* (250, 500, 1000, 2000, 4000 and 8000 $J_2$/plant) and *M. phaseolina* (0.25, 0.50. 1.0, 2.0, 3.0 and 4.0g of mycelial mat/plant). The results presented in Table 1 clearly showed that there was a significant reduction in plant growth characters particularly shoot and root length, fresh and dry weight of shoot and root at different inoculum levels with 1000 or more second stage juveniles/kg soil. These results expressing minimum pathogenic level (1000 $J_2$/kg soil) of *Meloidogyne javanica* on balsam. However, these findings are not in agreement with those of Khan *et al.* (2006) who reported that the damaging threshold level of *M. javanica* on balsam was 500 $J_2$/plant. The differences in the inoculum threshold levels might be due to the different races of *M. javanica*. At this level, symptoms like thinly spread foliage with small leaves, yellowing and premature shedding of leaves, and also stunting of plants, were also recorded. The economic threshold level of *Meloidogyne incognita* on balsam was also reported as 1000 $J_2$/kg

soil by Khan (2003). The damaging threshold level of *M. javanica*, on different crops ranged from 500 to 2000 $J_2$/kg soil (Swarup *et al.*,1989) The differences observed in damaging threshold level of root-knot nematode (*Meloidogyne* spp.) may be attributed to the differences in experimental conditions, the cultivars, used species and races of the root-knot nematode involved.

It was also observed that with an increase in the level of inoculum there was a progressive increase in host infestation as indicated by the number of galls as well as the population of nematodes. Moreover, the rate of nematode multiplication was reduced with the increase in the inoculum density of *M. javanica*. This might be due to the destruction of root system by the parasitism of root-knot nematode which led the competition for food and nutrition among the developing nematodes within the root system and also due to inability of juveniles to find out new infection sites for subsequent generation (Ogunfowora, 1977). The high rate of multiplication at low levels of inocula, on the other hand, could possibly be due to the positive factors like abundance of food, lack of competition and the ability of host to support these levels of population. According to Oostenbrink (1966), the increase in the nematode populations and the subsequent reduction in the yield of crop are directly influenced by the initial density of the nematodes in the soil. His view holds true with the present findings where in plant growth was proportionately affected with increase in the number of galls and final nematode population. The progressive decrease in plant growth and nematode multiplication with the increasing inoculums of root-knot nematode on different crops have also been reported by (Seinhorst, 1960, Raut and Sethi, 1980, Khan and Husain, 1989, Meena and Mishra, 1993, Patel *et al.*, 1996; Dalal and Bhatti, 1996; Pathak *et al.*, 2000; Khan, 2003; Khan *et al.*, 2004; Kumar and Pathak, 2005; Khan and Ashraf, 2006; Khan *et al.*, 2006a; Khan *et al.*, 2006b).

The inoculation of *Macrophomina phaseolina* at the rate of 0.25, 0.50 and 1.0g mycelial mat/plant did not show any significant variation in plant growth as compared to control. Moreover, a significant progressive decrease in plant growth characters were recorded at and above 2.0g mycelial mat/plant. Similarly, the root-rot caused by *M. phaseolina* was also increased with the increase in the inoculum levels except at the lowest inoculum.

It can the concluded from the above results that the damaging threshold level of *M. javanica* on balsam was 1000 $J_2$/plant and that of *M. phaseolina* was 2.0g mycelial mat/plant. The information gathered from the present study may provide the base line for further research to develop appropriate strategies for the management of root-knot nematode (*M. javanica*) and root-rot fungus (*M. phaseolina*) on balsam.

Balsam seedlings were inoculated with *M. javanica* and *M. phaseolina* either individually or concomitantly as well as sequentially with an interval of 15 days between the nematode or fungal inoculations to determine whether the interaction was concomitant or sequential. The greater reduction in plant growth characters were observed in the plants inoculated with *M. javanica* and *M. phaseolina* either concomitantly or sequentially as compared to their individual inoculation. The maximum reduction in plant growth characters were recorded in the plants inoculated with *M. javanica* 15 days prior to *M. phaseolina* followed by *M. javanica* + *M. phaseolina* and *M. phaseolina* 15 days prior to *M. javanica*.

The plant growth reduction in the first two treatments caused by the nematode and fungus together was greater than the sum of the independent effects, resulting in a synergistic interaction which was probably due to predisposition of plants by nematode to fungus attack such investigation involving with different nematode and fungus species have also been reported earlier (Caquil and Shephered, 1970; Kumar and Sivakumar, 1981; Carter. 1981; Khan and Husain, 1988 and 1999).

Although studies were not conducted to determine as to how the *M. javanica* predisposed balsam plants to fungus attack but stress and weakness of plants caused by prior nematode infection was probably the major predisposing factor. In addition nematode might have provided infection courts through which fungus entry might have been facilitated. Moreover, prior invasion of nematode into the roots thereby making the host more favourable for fungal infection by offering a metabolically rich substrate and/or nematode might also modify the rhizosphere thereby favouring the fungal growth.

Results from this study however, conflict with those of Gray *et al.* (1990) who found that survival of inoculated seedlings was smaller following a single inoculation with *P. megasperma* f. sp. *medicaginis* than following inoculation with *M. hapla* plus *P. megasperma* f. sp. *medicaginis*. Differences in the results of the two studies could

be due to the nematode and fungus competing for undifferentiated tissue in radicles of the newly germinated seed utilized in the Gray *et al.* (1990) study, limiting infection by both pathogens. Such competition may not have occurred in the present study. Since plants were 25 days old at the time of inoculation and considerable differentiated root tissue had developed. Hence, the nematodes would be expected to have invaded and parasitized the vascular tissue while the fungus invaded the cortical tissue.

Griffin *et al.* (1993) reported that the increase in nematode reproduction from preinoculation with *P. megasperma* f. sp. *medicaginis* in the greenhouse may be due to a partial breakdown of the cortical tissue by *P. megasperma* f. sp. *medicagnis* and a subsequent increase in the ability of the nematode to invade the vascular root tissue. Alternatively, *P. megasperma* f. sp. *medicagnis* may cause a physiological change in the root tissue that enhanced invasion and reproduction of *M. hapla.*

The extensive physiological and anatomical changes produce by infections of *Meloidogyne* and other species in the root system resulted in modification of the normal functions and possibly the defense mechanisms against soilborne pathogens. Gaint cells induced by *Meloidogyne* spp. are found often with higher infection sites of major and minor fungal soilborne pathogens and saprophytes (Mayoland Bergeson, 1970, France and Abawi, 1994). Bean plants infected by *M. incognita* contain significantly lower quantities of chlorophyll, carbohydrates, and nitrogen compounds, and show reduced water and nutrient uptake. Consequently such infected plants are weaker and support fewer flowers, pods and seeds as compared to healthy plants. Overall, plants infected with root-knot nematodes show increased value ability to any stress condition.

Lesser reduction in plant growth inoculated with the fungus followed by nematode understandable, as it is likely that by the time the plants were inoculated with the nematode the fungus got sufficient time to colonize the cortex making it less suitable for nematode attack or the fungus secretions produced adverse effects on nematodes, as observed in the hatching and mortality experiments and also reported earlier (James, 1966; Khan and Husain, 1989; Ashraf and Khan, 2005).

The number of galls and reproduction factor (R) of *M. javanica* was reduced in the presence of *M. phaseolina.* Detrimental effect of various fungi on nematode

populations has also been reported by earlier workers (France and Abawi, 1994: Chahal and Chahal, 1998 and Back *et al.*, 2002) and is ascribed to destruction of root tissues by the fungus this led to reduced root system in these treatments which was unable to support large number of galls consequently further reproduction of the nematode was arrested.

The extensive root damage due to root-rot caused by *M. phaseolina* probably decreased the sites for *M. javanica* penetration and sedentary feeding. Moreover, gaint cells induced by *Meloidogyne* spp. are known to increase susceptibility to the root-rot pathogen and soil microorganisms. This damage may also explain the restricted root-knot nematode reproduction potential and the development of root galling symptoms. In addition, translocatable toxic metabolites produced by plant pathogenic fungi may cause deterioration of gaint cells, reduce hatching, and immoblize the second-stage juveniles (Griffin *et al.*, 1993; France and Abawi, 1994). All these process may have contributed to the observed suppression in reproduction of *M. javanica* in this investigation. Contrarily, Seinhorst and Kumiyasu (1971), Prasad *et al.* (1980) and Varshney (1982) have reported significant increase in nematode population where the plants were inoculated with nematode and fungus in comparison to nematode alone. In addition, an increase in the final density of *Pratylenchus* spp. on different hosts (Mc Keen and Mountain, 1960; Michell and Powell, 1972) and of root-knot nematode on chrysanthemum (Johnson and Littrell, 1969) infected with vascular wilt fungi have also been reported.

It can be concluded from the above findings that the interaction between *M. javanica* and *M. phaseolina* on balsam was both concomitant and sequential (*M. javanica* 15 days prior to *M. phaseolina*) as these treatments showed a synergistic effect on the reduction of plant growth parameters as compared to the individual inoculation of either *M. javanica* or *M. phaseolina.* Similarly, the concomitant inoculation of the plants even with the lowest inoculum level i.e. 200 $J_2$ + 0.26g mycelial/mat caused synergistic effect on the reduction of plant growth parameters.

The results presented in Table-4 indicated that root-knot nematode *M. javanica* required 25 days to complete its life cycle on balsam. However, on the other hand, the *M. javanica* takes about 33 days to complete its life cycle on balsam in presence of fungus. The increased duration of life cycle of *M. javanica* might be due to the

adverse effect of *M. phaseolina*. In the previous experiments, it was observed that the root system got damaged in the plants inoculated with *M. javanica* and *M. phaseolina*, resulting in reduction of root weight and size. This may be the main reason for reduced juvenile penetration and consequently in the number of juveniles developing into females and in the number of eggs/eggmass in fungus inoculated plants. Further, the delay in the development and maturity of root-knot nematode when *M. phaseolina* was involved could probably be due to the interference in ecological balance inside the roots as a result of colonization by the fungus *M. phaseolina*. Instances of similar adverse effect of fungus on nematode life cycle have also been recorded by Ryder and Crittenden (1965), Littrell and Johnson (1969), Mahapatra and Swain (1999), Khan (2002) and Ashraf & Khan (2005). Production of more males in *M. javanica* and *M. phaseolina* inoculated plants could be due to nutrition stress resulting from fungal infection. Ketudat (1969) reported that fungi may be involved in nematode sex ratios. He found that the number of males of *Heterodera rostochiensis* in tomato increased when *Rhizoctonia solani, Verticillium albo-atrum* or gray sterile fungus was added. He further reported that male-female ratio increased with an increase in the amount of fungus inoculum. These results are also in agreement with those of Khan (2002) and, Ashraf and Khan (2005). Total duration of life cycle of *M. javanica* on different hosts has also been reported by various workers from time to i.e 30 days on carrot (Huang, 1986); 23 days on balsam (Khan *et al.*, 2006); 29 days on lettuce (Khan and Ashraf, 2005); 27 days on broccoli (Khan *et al.*, 2006); 28 days on brinjal (Ravi Chandra *et al.*, 1988). The total time taken for completion of life cycle of *Meloidogyne* species under optimum condition (optimum temperature 27-30$^0$C) was reported as 3-4 weeks by Parihar *et al.* (1998). The variation in time required to complete the life cycle might be due to the number of ecological factors especially host status, soil texture, temperature, moisture etc. (Wallace, 1973; Swarup and Dasgupta, 1986; Bhatti and Walia, 1992).

The fungal filtrate of root-rot fungus *M. phaseolina* was effective to a varying degree in killing the second stage juveniles and also inhibiting the hatching of *M. javanica*. The exposure time and concentration were important determinants for this respect. Fungal filtrates of some fungi were also found nematicidal against root-knot nematode and percentage mortality and inhibition of hatching was directly proportional to the concentration of fungal filtrates (Khan and Khan, 1992, Ashraf &

Khan, 2005). Khan and Husain (1989) reported that culture filtrate of ten soil fungi showed toxic effect against *Rotylenchus reniformis*. Percentage mortality of reniform nematode was directly proportional to the concentration of culture filtrate and exposure time to each filtrate. There are many reports showing nematicidal action of soil fungi besides inhibiting larval emergence of plant-parasitic nematodes due to presence of toxic metabolites in the fungal filtrates. (Mankau, 1969; Desai *et al.*, 1972; Alam *et al.*, 1973; Chhabra *et al.*, 1979; Khan *et al.*, 1984; Ghewande *et al.*, 1984; Arya and Saxena, 1988; Chahal, 1991).

The results presented in Table-6 showed the stimulatory effect galled root-extract on the growth and scloratial formation of *M. phaseolina* was directly correlated with the density of root-knot nematode infected the plants. This stimulatory effect of the galled root-extract on the growth and sclerotial formation of *M. phaseolina* might be due to the presence of higher concentration of carbohydrates reducing sugar, amino acid etc. in the galled roots than in healthy roots (Gonzalez, 1982). These finding indicate that nematode made the roots a better environment for fungal developments, perhaps most simply by increasing the nutrients supply available for fungus. My results are also in agreement with those of Broodie and Cooper (1964), Golden and Van Gundy (1975), Kleineke-Borchers, and Wyss (1982) and Noguera and Smits (1982).

# *Summary and Conclusion*

# SUMMARY AND CONCLUSION

The reduction in plant growth characters of balsam was directly proportional to the inoculum levels of *M. javanica* i.e. with increasing the inoculum levels from 250 to 8000 second stage juveniles of *Meloidogyne javanica* ($J_2$) per plant, there was a corresponding increase in the reduction of plant growth characters of balsam. However, the inoculum levels upto 500 $J_2$/plant did not show significant reduction in plant growth characters as compared to control. Although, the significant reduction in plant growth was recorded at and above 1000 $J_2$/plant. Further, it was observed that the reduction in plant growth was not significant between the inoculum levels of 2000 and 4000 $J_2$, and 4000 and 8000 $J_2$ / plant.

It was also observed that with an increase in the level of inoculum there was a progressive increase in host infestation as indicated by the number of galls as well as the population of nematodes. Moreover, the rate of nematode multiplication was reduced with the increase in the inoculum density of *M. javanica*.

The inoculation of *Macrophomina phaseolina* at the rate of 0.25, 0.50 and 1.0g mycelial mat/plant did not show any significant variation in plant growth as compared to control. Moreover, a significant progressive decrease in plant growth characters were recorded at and above 2.0g mycelial mat/plant. Similarly, the root-rot caused by *M. phaseolina* was also increased with the increase in the inoculum levels except at the lowest inoculum.

It can the concluded from the above results that the damaging threshold level of *M. javanica* on balsam was 1000 $J_2$/plant and that of *M. phaseolina* was 2.0g mycelial mat/plant. The information gathered from the present study may provide the base line for further research to develop appropriate strategies for the management of root-knot nematode (*M. javanica*) and root-rot fungus (*M. phaseolina*) on balsam.

Balsam seedlings were inoculated with *M. javanica* and *M. phaseolina* either individually or concomitantly as well as sequentially with an interval of 15 days between the nematode or fungal inoculations to determine whether the interaction was concomitant or sequential. The greater reduction in plant growth characters were observed in the plants inoculated with *M. javanica* and *M. phaseolina* either concomitantly or sequentially as compared to their individual inoculation. The

maximum reduction in plant growth characters were recorded in the plants inoculated with *M. javanica* 15 days prior to *M. phaseolina* followed by *M. javanica* + *M. phaseolina* and *M. phaseolina* 15 days prior to *M. javanica.*

The simultaneous inoculation of plants with different inoculum levels of nematode and fungus synergistically increased root-rot and reduction in plant growth characters with the increase in the inoculum levels of *M. javanica* and *M. phaseolina* as compared to their individual inoculation. It was interesting to note that, significant reduction in plant growth (12.5) was observed even when the seedlings were concomitantly inoculated with lowest inocula (200 juvenile of *M. javanica* + 0.25g fungus of *M. phaseolina*).

It can be concluded from the above findings that the interaction between *M. javanica* and *M. phaseolina* on balsam was both concomitant and sequential (*M. javanica* 15 days prior to *M. phaseolina*) as these treatments showed a synergistic effect on the reduction of plant growth parameters as compared to the individual inoculation of either *M. javanica* or *M. phaseolina.* Similarly, the concomitant inoculation of the plants even with the lowest inoculum level i.e. 200 $J_2$ + 0.26g mycelial/mat caused synergistic effect on the reduction of plant growth parameters.

The percentage of male formation of *M. javanica* was greater (12.5%) in presence of *M. phaseolina* as compared to when *M. javanica* was present alone (3.7%).

The life cycle of *M. javanica* on balsam was completed within 25 days, whereas, the duration of life cycle was adversely affected in the presence of fungus (*M. phaseolina*) and it takes about 33 days to complete the life-cycle i.e. presence of *M. phaseolina* delayed the life-cycle of root-knot nematode (*M. javanica*) by 8 days.

The different concentration of culture filtrate of *M. phaseolina* showed significant inhibition in the juveniles emergence of *M. javanica* to varying degree except in the S/100 and S/1000 concentrations. There was a relative decrease in the hatching of *M. javanica* with the corresponding increase in the concentration of culture filtrate and the exposure period. The rate of hatching inhibition in the S, S/2 and S/10 concentration was low in the beginning but appreciably increase with the increase in the exposure period. The highest significant inhibition in the hatching of root-knot nematode was recorded in 'S' concentration at 96 hours.

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